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SOIL SCIENCE

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Pages 234–235. In columns 8 and 9 read $\frac{\text{Soil}}{\text{Subsoil}}$ instead of "Soil 1." Units for both columns are *per cent*.

THE TRANSIENT NATURE OF MAGNESIUM-INDUCED TOXICITY AND ITS BEARING UPON LIME-MAGNESIA RATIO STUDIES

W. H. MacINTIRE AND J. B. YOUNG

University of Tennessee Agricultural Experiment Station

Received for publication February 1, 1923

INTRODUCTORY

In earlier experiments (23) it was found that heavy additions of basic precipitated magnesium carbonate depressed the growth of wheat in baskets. The $MgCO_3$ treatments at several rates were based upon indications by the Veitch method (33). Those baskets were discarded after the harvesting of the single wheat crop. Consequently, persistence of the initial toxicity was not determined. In a later experiment precipitated magnesium carbonate was added to eight cylinders of a 128-unit series which contained one of the three basket soils. This 128-unit series comprised 4 sub-series, viz., K, 1–32; L, 1–32; M, 1–32; and N, 1–32; of identical treatment but different tillage and cropping. Series K and N were seeded to cowpeas and tall oat grass, respectively, while series L and M were fallow, with and without cultivation. The hydrated magnesium carbonate treatments were equivalent to 8 tons of CaO per 2,000,000 pounds of soil. After analyses showed that each of the heavy applications of magnesium carbonate had been decomposed (23), all cylinders of series N were seeded to tall oat grass. For three successive seedings the grass died after reaching a height of about three inches (plate 1). A good growth, however, was secured in the equivalent parallels treated with burnt lime, hydrated lime, limestone and dolomite. Differing from the short-period basket studies, the cylinder experiments have been continued over a period of nearly ten years and show the effect of the large magnesium residuals upon later seedings to tall oat grass. Instead of continued toxicity, the effect of the non-carbonate magnesium residuals was soon found to be beneficial. This transition from toxicity to fertility and possible reasons for it are to be considered in this contribution. The bearing of the findings upon the interpretations of earlier studies concerning the hypothesis of an optimum lime-magnesia ratio will also be considered in brief.

In the earlier work, with both baskets and cylinders, only residual carbonates were determined. It was not known whether the depressive influence on the wheat and the tall oat grass was due to an excess of magnesium ions, to an unfavorable hydroxyl-ion concentration, or to an unfavorable balance between calcium and magnesium ions, with or without relationship to the ions of other bases. Whatever the cause of the initial toxicity, it is

certain that the excess of magnesium or the concentration of hydroxyl-ions causing it was derived not from the original precipitated carbonate but from the hydrolysis of the recently precipitated silicate complexes. The increased supplies of magnesium nitrate and magnesium sulfate, resulting from additions of the carbonate, were a factor of possible importance.

Hydrogen electrode studies on the unleached basket soils were conducted by Plummer in 1917. He states (29) that, "The samples which have been treated with excessive amounts of calcium and magnesium carbonates show a greater concentration of OH than H ions in solution. . . . . Obviously, the new compounds of calcium and magnesium, whatever they may be, give a strong basic reaction."

EXPERIMENTAL

In addition to treatments of precipitated $MgCO_3$, the cylinder experiment also called for equivalent additions of CaO, $Ca(OH)_2$, $CaCO_3$, ground limestone, and ground dolomite. The magnesium carbonate treatments were made only on the 8-ton CaO-equivalent basis. Comparisons will be made, therefore, with the other materials only as they were chemically equivalent. The same loam soil was used in all 128 cylinders. Description of soil, cylinders, and treatments has been given in a previous contribution (23). The cylinders were placed and treatments made June 11, 1913. Eight weeks later the magnesium carbonate had disappeared and the soil was thus devoid of solid-phase added carbonate after this time (23). Two of the eight magnesium carbonate cylinders, one treated with magnesium carbonate alone and the other with carbonate and manure were seeded to Japan clover, April 7, 1914. Two corresponding cylinders were seeded to tall oat grass on the same date. The remaining four cylinders were left fallow. Because of failure to secure and maintain a stand of Japan clover in the summer, as well as vetch during the winter, especially in the unlimed controls. the clover was later discarded in favor of cowpeas.

YIELDS OF TALL OAT GRASS

A good stand of this crop was secured on all of the lime-treated cylinders seeded in April, 1914. However, chlorosis appeared in the growth of the grass on the magnesium carbonate cylinders N-31 and N-32, and gradually every seedling died, apparently as soon as the nutrient of the mother seed had been utilized. These two cylinders were twice re-seeded with the same result. They were then left fallow until October 26, 1914, when seed from an untreated cylinder was used for the fourth seeding. No cuttings of tall-oat grass were made from any of the cylinders during the fall of 1914, but several of the plants of the check cylinder 30, had produced sufficient seed to provide for the re-seeding of no. 31 and 32. The grass was harvested from all the N-series cylinders. those seeded to grass, on June 9, 1915 and a second crop

was harvested in the following fall. These and subsequent yields together with those from chemically equivalent additions of ground limestone and ground dolomite are given in table 1. Growth is shown in plates 2 and 3.

The yields from ground limestone and dolomite are very nearly parallel, both with and without manure. There is no evidence of toxicity from the large amounts of magnesium along with calcium in its native condition.

Comparing cylinders 18 and 32, the precipitated $MgCO_3$ shows a beneficial effect throughout the first five years, in spite of the delayed benefit. The difference is emphasized during the last two years because of the disappearance

TABLE 1

Yield of tall-oat grass in cylinders receiving different treatments, all being chemically equivalent to 28,180 lbs. of $CaCO_3$ per 2,000,000 lbs. of soil

SERIES N CYLINDER NO.	TREATMENT PER 2,000,000 LBS. OF SOIL	AIR-DRY WEIGHT								
		1915		1916	1917	1918	1919	1920	1921	Totals
		1st crop	2nd crop							1915 to 1918 / 1915 to 1921
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm. / gm.
18	30 tons manure	309.4	127.6	67.0	92.5	185.0	81.0	.....	.....	781.5 / 862.5
21	48 tons manure $CaCO_3$	368.5	158.2	264.0	116.5	207.0	168.0	254.0	345.0	1114.2 / 1881.2
27	$CaCO_3$	293.7	130.1	232.0	131.8	220.0	186.0	222.0	302.0	1007.6 / 1717.6
30	Nothing	229.5	117.4	144.0	91.8	196.0	65.0	92.0	34.0	778.6 / 969.7
31	$MgCO_3$	21.0*	234.7†	22.0	71.5	147.0	70.0	128.0	164.0	496.2 / 858.2
32	30 tons manure; $MgCO_3$	284.6	193.9	277.0	174.5	249.0	187.0	258.0	307.0	1179.0 / 1931.0
28	Limestone	308.8	137.8	242.0	141.3	217.0	76.0	259.0	297.0	1046.9 / 1678.9
29	Dolomite	281.9	122.5	225.0	139.5	265.0	90.0	233.0	242.0	1033.9 / 1598.9
22	48 tons manure; limestone	367.8	142.9	251.0	107.0	222.0	165.0	273.0	354.0	1090.7 / 1882.7
23	48 tons manure; dolomite	381.6	141.7	226.0	110.5	167.0	170.0	270.0	355.0	1026.8 / 1821.8

* Poor stand. Re-seeded after harvesting first crop.

† Good season after re-seeding.

of the stand in the former cylinder. The yield from the magnesium-carbonate-manure cylinder also is greater than that obtained from the precipitated calcium carbonate cylinders, either alone or when supplemented by manure, for both the initial 5-year period and the full 7-year period.

In cylinder 31, the first crop of 1915 was still subject to the toxic influence of the *absorbed magnesium carbonate.* It was again seeded, yielding from this second seeding a second crop in 1915 even greater than that produced by its companion cylinder 32, of the carbonate-plus-manure treatment. But a recurrent toxicity again depressed the growth as a result of the direct or indirect influence of the absorbed magnesium carbonate, in the absence of

manure, during 1916 and 1917. In 1917, the depression was less marked and a beneficial influence is shown during the last 3 years, resulting in a marked decrease in the disparity for the 7-year interval, as compared with the initial 5-year period. In fact, the plants which survived after the second crop of 1915 were decidedly more vigorous than those of the control, though fewer in number. The non-carbonate residues from the magnesium carbonate may have produced such changes in the quantity of available nitrate nitrogen and sulfate sulfur as to make them controlling factors in growth after the initial croppings. Occurring after the disappearance of the carbonate, the prevalence of soluble salts and their tendency to leach would cause osmotic changes which might have the effect suggested by Graves and Lund (7). It is evident, therefore, that: (a) Heavy applications of magnesium carbonate, both with and without manure caused a distinct initial toxicity to tall oat grass; (b) the toxic effect was still present at the end of one year, but was not consistently effective 18 months after the addition of carbonate and manure; (c) the toxicity was not due to the presence of magnesium carbonate, for it had disappeared 8 months prior to the first seeding; (d) a beneficial influence appeared in time, as the direct or indirect result of the substances derived from the addition of magnesium carbonate.

YIELD OF COWPEAS

The first seeding of cowpeas was made to series K June 16, 1917, or three years after the treatmentrs were applied. Two of these 32 cylinders, received heavy applications of precipitated magnesium carbonate. Table 2 shows that an increased growth of cowpeas, during each of the years 1917, 1918, and 1919 resulted from the additions of the hydrated precipitated carbonate of magnesium. The superiority of the green growth of cylinder 31, which received $MgCO_3$ over that of the control was much more striking in appearance than is indicated by the air-dry weight of crops. The cylinder which received precipitated magnesium carbonate and manure apparently produced a much heavier growth than the one which received magnesium carbonate only (plate 4).

The $MgCO_3$ treatments, both with and without manure, were responsible for a deep rich green color and an abundance of pods. The $MgCO_3$ without manure resulted in a yield more than double that secured from the control and also that from the parallel of calcium carbonate without manure. It also gave greater yields than were obtained from either limestone or dolomite alone, though the reverse was true in the same comparison where manure also was applied. The yield from the 30-ton manure treatment was less than that obtained from $MgCO_3$, or from carbonate and manure. However, the large residue of unfixed precipitated calcium carbonate, with 48 tons of manure, gave a larger yield than the absorbed magnesium carbonate and 30 tons of manure.

It is surprising to note that the total dry weight from cylinder 31, $MgCO_3$ alone, was greater than that from cylinder 32, wherein the supplementary treatment of manure was made; for, as is indicated by plate 4, the growth of stems, leaves and pods in the latter instance appeared much heavier. The manure apparently induced a much greater yield, in harmony with the yields from precipitated carbonate, limestone and dolomite alone, as compared with the respective treatments carrying manure. In spite of the appearance to the contrary, however, the data of each of the years 1917, 1918, and 1919 show a greater yield for the $MgCO_3$ alone, than for the carbonate plus manure.

If the amount of magnesium extracted from the non-carbonate residuals in the soil were the primary factor in causing toxicity, the manure additions would be expected to have intensified the toxic effect by increasing the amount

TABLE 2

Yield of cowpea hay in cylinders variously treated, all treatments being chemically equivalent to 28180 lbs. of $CaCO_3$ per 2,000,000 lbs. of soil

SERIES K CYLINDER NO.	TREATMENT PER 2,000,000 LBS. OF SOIL	YIELD OF HAY, AIR-DRIED			
		1917	1918	1919	Total
		gm.	*gm.*	*gm.*	*gm.*
18	30 tons manure	309.3	299.5	134.5	743.3
21	48 tons manure; $CaCO_3$	310.8	442.3	240.5	993.6
27	$CaCO_3$	218.8	40.5	83.5	342.8
30	Nothing	164.3	221.8	34.5	420.6
31	$MgCO_3$	261.8	307.0	302.0	870.8
32	30 tons manure; $MgCO_3$	250.0	260.5	291.0	801.5
28	Limestone	161.0	280.3	153.5	594.8
29	Dolomite	139.8	192.3	266.5	598.6
22	48 tons manure; limestone	356.0	472.0	344.0	1172.0
23	48 tons manure; dolomite	388.3	401.0	461.8	1251.1

of magnesium available to the plant, unless a corrective balance were maintained by parallel increases in the amounts of the other nutrients supplied either directly or indirectly by the manure. For, the free water of the soil would have become richer in CO_2 derived from the added organic matter, and being a stronger solvent it would have caused a more extensive hydrolysis of the non-carbonate magnesium residuals.

In agreement with the tall-oat grass yields, the cowpeas from the dolomite treatments, with and without manure, fail to show any toxic effect of the magnesium of the dolomite, nor any inferiority to those of the limestone treatments.

Since it was not known, in the case of the cowpeas, whether an initial and transient toxicity would have preceded the fertility of the later period, as in the case of the tall-oat grass, information on this point was sought.

INITIAL TOXICITY OF $MgCO_3$ ON COWPEAS IN WAGNER POTS

The same soil used in the cylinders was taken for a series of pots in experiments to determine any initial toxicity of magnesium toward cowpeas. However, the plot from which the pot soil was taken had been lightly limed twice previously within a period of 14 years, and small specks of unabsorbed calcium carbonate were still visible. Four Wagner pots were used, each containing 7,150 gm. of air-dry soil. To each of three of these was added 99.3405 gm. of analyzed precipitated $MgCO_3$, equivalent to 28,180 pounds of $CaCO_3$ per 2,000,000 pounds of soil. The $MgCO_3$, used was a composite of several samples obtained from the California, New York, Mississippi and Missouri Experiment Stations. The control pot received no $MgCO_3$. In order to insure against potash deficiency, each pot received 0.4250 gm. of KCl. The treatments were added August 2, 1918. The following day, the soil was brought to a moisture content of 30 per cent, on the air-dry basis and the cowpeas were sown to the control and to one treated pot. The moisture content was then permitted to drop to 20 per cent, and there maintained during the experiment. The original air-dried soil contained 0.072 per cent CO_2 which was increased to 0.415 per cent after the addition of hydrated magnesium carbonate. Subsequent analyses of sun-dried freshly sampled soil showed a CO_2 content of 0.259 per cent on August 14, 0.261 per cent on August 29, and 0.255 per cent on September 24, indicating a distinct retardation of the initial rapid rate of $MgCO_3$ absorption, as influenced by the two previous additions of burnt lime.

Eight seeds were sown in the control and in one $MgCO_3$-treated pot. However, since all but two of the seeds died in the treated pot shortly after sprouting, the plants in the control were thinned to the same number. By September 6 chlorosis was so severe that it became necessary to harvest the plants in order to secure satisfactory photographs (plate 5, fig. A). The second seeding was made to another treated pot and control September 24, 1918; the plants were thinned as formerly and the crops were harvested and photographed October 23. As indicated by plate 5, fig. B, the initial toxicity was still pronounced. The control and the third magnesium-treated pot were then kept moist without leaching, until September 3, 1919, thirteen months after the treatment, when they were seeded to cowpeas from the same lot of seed previously used. Thirty days later the plants, thinned to five, were harvested and photographed with the result shown in plate 5, fig. C. The green weight of the plants from the control was 19.07 gm. as against 24.40 gm. for those in the treated pot. The color of the plants grown in the soil treated with magnesium carbonate was much deeper than that of the control plants. It is apparent, therefore, that without leaching the aging of the magnesium additions in moist soil greatly lessened the initial toxicity, if it did not convert it to fertility. From the growth in the Wagner pots it seems reasonable to conclude that the magnesium carbonate additions in the cylinders would have been toxic to the cowpeas as in the case of the tall oat grass, had the cowpeas and grass been sown at the same time.

THE ACCUMULATION OF WATER-SOLUBLE SALTS OF CALCIUM AND MAGNESIUM IN THE WAGNER POTS

Lysimeter investigations upon the soil used in both cylinders and Wagner pots demonstrated that the heavy treatment of precipitated $MgCO_3$ had caused an extensive loss of both nitrates and sulfates during the first year after treatment. It appeared then that the cowpea growth in the Wagner pots took place in spite of the presence of a large amount of these two soluble salts of magnesium. The lysimeter results also indicated that the maximum production of the magnesium salts had taken place prior to the final tall oat grass seeding, which was a year after treatment. But, notwithstanding the leaching from the cylinders during the first 12 months after treatment, and the absence of any residual carbonate during the latter 10 months of the period, the first three seedings of tall oat grass had been killed off.

TABLE 3

Soluble calcium in soil from two Wagner pots at time of harvesting cowpeas

ZONE	TREATMENT	IN 120 GM. OF AIR-DRY SOIL			$CaSO_4$ PER 2,000,000 LBS. AIR-DRY SOIL, IF ALL Ca WERE PRESENT AS $CaSO_4$
		Determined as CaO	$CaSO_4$—if present as sulfate		
		gm.	*gm.*	*per cent*	*lbs.*
Upper	None	0.0155	0.0376	0.0321	642
	$MgCO_3$	0.0067	0.0163	0.0139	278
Middle	None	0.0157	0.0381	0.0324	648
	$MgCO_3$	0.0057	0.0138	0.0118	236
Lower	None	0.0147	0.0357	0.0304	608
	$MgCO_3$	0.0049	0.0119	0.0101	202
Average	None	0.0153	0.0371	0.0316	632
	$MgCO_3$	0.0058	0.0141	0.0119	238

The soil of the unleached Wagner pots, used in the observations of initial toxicity, was subjected to water extraction analysis to determine soluble calcium and magnesium salts present at the period when toxicity was no longer evidenced by cowpeas. The soil was removed in three $2\frac{2}{3}$ inch layers, which will be spoken of as the upper, middle, and lower zones; 750 gm. of soil from each zone was mixed with 3,500 cc. of distilled water, the suspensions agitated for 8 hours, permitted to stand overnight and filtered. Three liters of the filtrate were concentrated and aliquots used in determinations of calcium, magnesium and sulfates. The results given in tables 3, 4 and 5, and titrations show that the recovered bases were present as neutral calcium and magnesium salts engendered and accumulated over the 14 month interval of moist contact, rather than the relatively insoluble carbonate forms. The

extraction aliquots from 120 gm. fractions gave recoveries of 0.0155 gm., 0.0157 gm. and 0.0147 gm. of CaO, table 3, from the upper, middle and lower zones of the control pot represent respectively 642 lbs., 648 lbs. and 608 lbs., per 2,000,000 lbs. of air-dry soil, if the dissolved calcium was present as

TABLE 4

Soluble magnesium in soil from two Wagner pots at time of harvesting cowpeas

ZONE	TREATMENT	IN 120 GM. OF AIR-DRY SOIL			$MgSO_4$ PER 2,000,000 LBS. AIR-DRY SOIL, IF ALL Mg WERE PRESENT AS $MgSO_4$
		Determined as $Mg_2P_2O_7$	$MgSO_4$—if present as sulfate		
		gm.	*gm.*	*per cent*	*lbs.*
Upper	None	0.0078	0.0084	0.0072	144
	$MgCO_3$	0.1104	0.1194	0.1078	2156
Middle	None	0.0067	0.0072	0.0061	122
	$MgCO_3$	0.1224	0.1324	0.1129	2258
Lower	None	0.0060	0.0065	0.0055	110
	$MgCO_3$	0.1119	0.1210	0.1031	2062
Average	None	0.0068	0.0074	0.0063	126
	$MgCO_3$	0.1149	0.1243	0.1079	2158

TABLE 5

Soluble sulfates in soil from Wagner pots at the time of harvesting cowpeas

ZONE	TREATMENT	IN 120 GM. AIR-DRY SOIL					SULFATES IN 2,000,000 LBS. AIR-DRY SOIL	
		$BaSO_4$ determined	$CaSO_4$ equivalent		$MgSO_4$ equivalent		If present as $CaSO_4$	If present as $MgSO_4$
		gm.	*gm.*	*per cent*	*gm.*	*per cent*	*lbs.*	*lbs.*
Upper	None	0.0486	0.0283	0.0241	0.0251	0.0213	482	426
	$MgCO_3$	0.0646	0.0377	0.0321	0.0333	0.0283	642	566
Middle	None	0.0355	0.0207	0.0177	0.0183	0.0156	354	312
	$MgCO_3$	0.0386	0.0225	0.0192	0.0199	0.0190	384	380
Lower	None	0.0321	0.0187	0.0159	0.0166	0.0142	318	284
	$MgCO_3$	0.0379	0.0221	0.0188	0.0195	0.0166	376	332
Average	None	0.0387	0.0226	0.0193	0.0200	0.0170	386	340
	$MgCO_3$	0.0470	0.0274	0.0233	0.0242	0.0206	466	412

calcium sulfate. These calcium recoveries and the much smaller amounts of soluble magnesium recovered from the control demonstrate that nitrate and sulfate generations were cared for mainly by native calcium rather than native magnesium. On the other hand, the calcium recoveries from the

magnesium treatment were decidedly less than those from the control. This shows that nitrates and sulfates engendered in the magnesium-treated pot were neutralized by the absorbed magnesia, which in this way exerted a protective action upon the native soil calcium.

The large gains in soluble magnesium recovered from the magnesium treatment in all zones show that more than 2000 pounds of magnesium sulfate equivalent would have been accounted for upon the assumption that this salt was leached. However, the sulfate determinations and hypothetical combinations of table 5, and the fact that only small amounts of chlorine were leached made it necessary to conclude that most of the magnesium losses were due to extractions of magnesium nitrate. This agrees with unpublished lysimeter data upon the same soil and treatment which showed the hydrated magnesium carbonate to be very active in increasing leachings of nitrates.

If all of the sulfate radical were combined with calcium, there would be no great excess of calcium sulfate and the differences between the amounts in the several zones would not be great; nor would the average amount of the control differ greatly from that of the magnesium carbonate treatment, 386 lbs. as against 466 lbs. The same statements hold in the case of the assumption that all soluble SO_4 was combined with magnesium, the averages of the control and the magnesium-addition pots being 340 lbs. and 412 lbs., respectively, per 2,000,000 lbs. of soil. However, in spite of the appreciable quantities of sulfates and the larger quantities of magnesium nitrate, the cowpeas were growing at the time of harvest and soil analysis, without any apparent indication of the toxicity, which was so marked in the two first plantings. The amounts of soluble salts formed in the unleached pots could hardly be as great during the first 30 days after treatment, nor during the first eleven weeks, as were the amounts which accumulated during the longer period of 14 months. Yet, toxicity was manifested in the case of the peas growing during the first 30 days and in the case of those grown during a 30-day period which began 51 days after the incorporation of the magnesium carbonate, though it was not indicated in the 30-day period between the thirteenth and fourteenth months after treatments, at which time the excessive accumulations of neutral salt were found by the water extractions.

Tolerance to the magnesium ion could not have been established under the conditions of the pot experiments and yet, coincidently with probably greater soluble-salt accumulation over that present initially, the toxic influence apparently had been dissipated. This would indicate rather strongly that a change in reaction had taken place. Thus, initially, there probably occurred a smaller accumulation of neutral and soluble magnesium salts and a larger hydroxyl-ion concentration; and later, a larger occurrence of the neutral magnesium salts, accompanied by a decrease in hydroxyl-ion concentration. An accumulation of the neutral salts of the magnesium would tend to depress, or force back, the dissociation of the basic salts whereby an excess of hydroxyl-ions would less readily occur. While this might account for the

dissipation of the toxicity in the case of the unleached Wagner pots, it would hardly apply in the case of the field cylinders where leaching was in effect. It may be that the hydroxyl-ion concentration was reduced in the cylinders after the early disappearance of the more readily dissolved residual carbonate, together with the retardation of hydrolysis, because of the applied magnesium becoming more firmly absorbed and fixed. This question was not involved in the result sought in the original planning of the cylinder experiments; how-

TABLE 6

pH values of distilled water extracts of cylinder soils previously treated with precipitated carbonates of calcium and magnesium—extractions obtained by agitation and over night contact. Ratio of soil to water was 1 to 25

TREATMENT APPLIED JUNE, 1913*	DATE OF SAMPLING AND BRINGING TO AIR-DRY CONDITION	INDICATORS USED AND DETERMINATIONS OBTAINED MARCH 10, 1922				
		Methyl red, range 4.4–6.0	Brom-cresol purple, range 5.2–7.0	Brom-thymol blue, range 6.0–7.8	Phenol red, range, 6.8–8.6	Cresol red, range 7.2–9.0
None†	May 6, 1918	→	5.9	←	←	←
None‡	June 18, 1918	5.2	5.6	←	←	←
$CaCO_3$‡	June 18, 1918	→	6.5	6.8	7.1	←
$MgCO_3$‡	Aug. 15, 1913	→	6.0	6.2	←	←
$MgCO_3$‡	May 6, 1918	5.9	5.6	←	←	←

* All additions equivalent to 28180 lbs. $CaCO_3$ per 2,000,000 lbs. of soil.

† Composite of 4 cylinders.

‡ Composite of 8 cylinders.

TABLE 7

pH values of carbonated water extracts of cylinder soils, previously treated separately with precipitated carbonates of calcium and magnesium—extractions obtained by agitation and overnight contact. Ratio of soil to carbonated water 1 to 25.

TREATMENT APPLIED JUNE, 1913*	DATE OF SAMPLING AND BRINGING TO AIR-DRY CONDITION	INDICATORS USED AND DETERMINATIONS OBTAINED MARCH 10, 1922			
		Brom-phenol blue, range 3.4–4.8	Methyl-red, range 4.4–6.0	Brom-cresol, purple, range 5.2–7.0	Brom-thymol blue, range 6.0–7.8
None†	May 6, 1918	→	5.1	←	←
None‡	June 18, 1918	→	5.0	←	←
$CaCO_3$‡	June 18, 1918	→	5.5	←	←
$MgCO_3$‡	Aug. 15, 1913	→	5.6	←	←
$MgCO_3$‡	May 6, 1918	→	5.4	←	←

* All additions equivalent to 28180 lbs. $CaCO_3$ per 2,000,000 lbs. of soil.

† Composite of 4 cylinders.

‡ Composite of 8 cylinders.

ever, it was thought well to determine the pH values of solutions of the composite samples used also for the analytical work reported in tables 10, 11, and 12. These values are given in tables 6 and 7.

The laboratory samples taken in the early part of the initial year and at the close of the five-year period were used. They had been preserved in air-

dry condition. The extract were made with both distilled and carbonated water, using 1 gm. soil to 25 cc. water with overnight contact. The method used was that of Van Alstine (32).

Distilled water extracts: The distilled water extracts give pH values in each case below those of neutrality. The highest value is that derived from the calcium carbonate treatment and in this instance an excess of carbonate is present in the soil. The pH value of the magnesium-treated carbonate-free sample of August 15, 1913, taken 8 weeks after treatment is higher than that of the same soil sampled May 6, 1918. The soil, which remained subject to both leaching and continued dynamic influences while moist, is therefore of lower pH value than the soil which was more quickly brought to the air-dry state and kept in that condition.

Carbonated water extracts: The carbonated water extracts show a less pH value in each case than was shown by the distilled water extracts. A slight difference is shown between the values given to the two samplings of the magnesium carbonate additions, the first of which is slightly nearer, and the second slightly farther from neutrality than the reading secured from the calcium carbonate treatment which still carries a carbonate excess, as contrasted to the absence of residual carbonate in the case of the magnesium carbonate additions. Toxicity may have been induced, therefore, by readily soluble magnesium complexes shortly after their fixation, a progressive change in pH values following parallel with the aging of the fixed base.

POSSIBLE REASONS FOR THE INITIAL TOXICITY AND ITS SUBSEQUENT DISAPPEARANCE

The age of a precipitate is one factor which influences its solubility coefficient. The carbonate-silicate study of MacIntire and Willis (22) has shown that an excess of magnesium carried by an aged and complex silicate, serpentine, was not toxic when applied in heavy amounts to the Cumberland loam used in the cylinders and lysimeters. We have shown also (19, 23) that the silicate reaction may be reversed with an appreciable quantity of magnesium entering into the carbonated water solution, which bathes the mass of soil containing freshly precipitated siliceous complexes. The finely ground natural serpentine, however, is but slightly acted upon by the same agency under similar conditions. Since it was known that the magnesium carbonate had become fixed, or silicated, prior to the tall oat grass seeding, as established by carbonate determinations, and that the greater part of the applied magnesium was still present in the soil, as indicated by data obtained from analyses of lysimeter leachings, it seemed logical to assume that the newly formed siliceous compounds had progressively passed into more complex and more stable complexes. Such an hypothesis would suggest that the fixed magnesium would have a decreasing tendency to hydrolyze. Were the basic components of the freshly formed magnesium complexes to undergo progressive decrease in their total amounts through leaching, while remaining of constant proportions, they could be

reasonably expected to decrease in solubility, or tendency to undergo hydrolysis and re-carbonation in the free carbonated water of the soil. It is possible, however, under the dynamic conditions inherent to the soil mass, that the several basic elements leached out in different proportions and that successive progressive complexities and changes in proportion of bases would ensue, thereby converting the added magnesium into combinations more firmly fixed.

Thus, applying the related lysimeter and other studies to the cylinder results, there was probably an abundance of magnesium forced upon the growing plants in the cylinders, not only as magnesium bicarbonate, but also as the biological end-products, nitrate and sulfate, as a result of accelerated nitrification and sulfofication induced by the bicarbonate derived from the ready hydrolysis of the freshly precipitated silicates. Although Kelly (9) showed, in incubation studies, that basic magnesium carbonate caused a distinct retarding of nitrification of added nitrogenous materials, we have abundant data to the opposite effect, in so far as native nitrogenous substances are concerned. Sulfofication, as measured by accelerated outgo of sulfates, was also found to have been greatly increased by magnesium additions to the same loam in the lysimeters.

With the aging process continued, and after passage of the more readily soluble salts down into the subsoil, the soil medium apparently approached the normal, with a diminution of the intense initial toxicity. Such an explanation would explain in a measure the initial phenomenon, whether the cause be attributed to an excess of magnesium ions or to an excessive hydroxyl-ion concentration.

An accumulation of magnesic salts may have caused changes in osmotic pressure, particularly during the earlier periods. Ewart (3) studied the influence of high salt concentrations in the soil solution and found a distinct retarding in germination of seed. While a splendid germination was secured from our seeding of tall oat grass, it is quite possible that osmotic pressure had some effect upon growth.

Viewed from another angle, the toxicity might be considered as the result of disrupted equilibria, if extensive basic interchange were assumed. Assuming that magnesium carbonate liberated and caused excessive leaching of calcium, sodium, and potassium from their native combinations, such basic replacement during the period of greatest transformation of magnesium carbonate into non-carbonate forms would tend toward beneficial antagonisms rather than an excess of magnesium and a paucity of calcium and the alkalis. We have data in manuscript form which bears directly upon such an assumption for this particular soil. But, the toxicity noted prevailed much longer. Furthermore, the writer (20) has shown that if sodium and potassium are liberated by calcium and magnesium in this loam, the liberated alkalis do not pass into the free water of the leachings. This finding is in harmony with that of Lyon and Bizzell (17, 18). Nevertheless, enough soluble magnesium salts would still be available to maintain a high ratio of magnesium to calcium. This would tend

to maintain toxicity unless the plant were to become magnesium tolerant. But, instead of initial fertility and subsequent toxicity the reverse order was definitely established for tall oat grass and also, later, for cowpeas. The temporary toxicity to tall oat grass and the subsequent fertility when leaching was permitted under normal field conditions, the same tendency later in the basket work with cowpeas where no leaching took place, the fact that toxicity ceased although most of the added magnesium was still in the soil and with pH values undetermined seem to justify the conclusion that the residual magnesic materials had undergone some decided change in parallel to the occurrence of initial toxicity and subsequent fertility.

LOSSES OF MAGNESIUM DURING THE 5-YEAR PERIOD

One year after the beginning of the cylinder studies, a 46-unit lysimeter equipment was installed, the same loam being used in both cylinders and lysimeters. All of the basic materials used in the cylinders were also used in the lysimeters in amounts equivalent to 8 tons of CaO per 2,000,000 pounds of soil. The only difference between the methods of incorporating materials was that the materials were mixed throughout the upper 6 of the 8 inches of soil in the cylinders, while lysimeter treatments were mixed throughout the full 8-inch depth. The losses from the cylinders from 1913–1917 are taken as comparable to those from the lysimeters during 1914–1918.

The data of table 8 also show the amounts of surface soil leachings of magnesium and calcium salts stopped by the subsoil. The heavy loss of 1914–1915, equivalent to 42 per cent of the 8-year total resulted from an annual rainfall of 47.96 inches. Since a large part of the magnesium added was still present in the soil in 1919, the decreased leaching indicates that the portion still held by the soil had decreased in solubility. Comparing tank 4 with tank 25, it appears that the foot of subsoil stopped, in round numbers, 3,300 lbs., 1,400 lbs., 1,100 lbs., 400 lbs. and 500 lbs. for the first five years, respectively. The same order of differences will be noted for the MgO-treatments. Each annual surface soil outgo of magnesium from the $MgCO_3$ treatment was respectively greater than the amount of calcium lost by the corresponding calcium treatment. The same relaton also holds true in a similar comparison between MgO and CaO, in the surface soil tanks. This obtains, although the disparity between the respective solubilities of precipitated magnesium carbonate and calcium carbonate in carbonated water was offset by the far greater speed in the absorption, or fixation, of the magnesium carbonate by the soil.

A previous contribution (23) and table 10 show that the solubility of the residual calcium carbonate from a heavy treatment is greater than that of the residual absorbed non-carbonate magnesian compounds, when the treated soil is extracted with carbonated water. But, it is quite possible that another factor may be involved where the CO_2 of the soil water is derived from that generated within the soil. Since combinations of magnesium were more active

TABLE 8

Amounts of $MgCO_3$ and $CaCO_3$ leached from 8-ton treatments of 93.0 per cent CaO, and equivalences of MgO, $CaCO_3$ and $MgCO_3$ per 2,000,000 lbs. of soil in terms of $CaCO_3$ equivalence as pounds per acre. 1914–1919

TANK NUMBER	TREATMENT	AS TO SUBSOIL	1914–1915			1915–1916			1916–1917			1917–1918			1918–1919			TOTAL 1914–1919		
			$CaCO_3$	$MgCO_3$	Total	$CaCO_3$	$MgCO_3$	Total	$CaCO_3$	$MgCO_3$	Total	$CaCO_3$	$MgCO_3$	Total	$CaCO_3$	$MgCO_3$	Total	$CaCO_3$	$MgCO_3$	Total
			lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	CaO	None	1767	112	1879	1062	61	1123	1009	36	1045	446	39	485	732	44	776	5016	292	5308
22	CaO	1 foot	499	279	778	408	300	708	410	323	733	197	165	362	305	260	565	1819	1327	3146
2	MgO	None	180	2274	2454	74	1346	1420	164	1448	1612	61	570	631	123	817	940	602	6455	7057
23	MgO	1 foot	439	239	678	477	361	838	442	374	816	195	252	447	306	240	546	1859	1466	3325
3	$CaCO_3$	None	1406	149	1555	831	58	889	913	74	987	453	48	501	749	48	797	4352	377	4729
24	$CaCO_3$	1 foot	350	184	534	350	250	600	435	356	791	174	205	379	383	301	684	1692	1296	2988
4	$MgCO_3$	Note	189	3525	3714	79	1791	1870	145	1569	1714	56	631	687	113	787	900	582	8303	8885
25	$MgCO_3$	1 foot	388	210	598	470	338	808	551	480	1031	180	231	411	293	280	573	1 882	1539	3421

than those of calcium upon the soil flora, as determined by the leached nitrate and sulfate end-products, it is quite possible that compounds of magnesium may also cause a greater generation of CO_2 from soil organic matter and produce a more strongly carbonated soil-water with a greater solvent action than that induced by calcareous materials. Again, it is quite possible that with equal generation of CO_2 the magnesic compounds may materially depress a soil's retentive properties for CO_2 causing it to yield that gas more readily to the free water of the soil.

As applied to the cylinder studies upon plant growth, these observations strengthen the hypothesis that the disappearance of the toxicity induced by the non-carbonate residuals derived from the applied magnesium was not caused so much by its elmination through leaching, as by the fact that the residues in the cylinders were not so active and therefore possessed of less tendency to produce those conditions which proved toxic to plant growth. The data do not determine, however, whether the apparently diminished activity and the disappearance of toxicity were caused by a lessened production of magnesium nitrate, or magnesium sulfate—excesses of magnesium-ions—or to a diminished hydroxyl-ion concentration, but the probability of the latter was indicated by the pH values given in tables 6 and 7.

CONCERNING COMBINATIONS IN WHICH SOLUBLE MAGNESIUM MAY HAVE LEACHED FROM THE CYLINDERS

While the data of table 8 set forth the total amounts of calcium and magnesium leached from the magnesium carbonate and equivalent magnesium oxide treatments, as well as the same losses from parallel and equivalent treatments of both carbonate and oxide of calcium, they do not specifically determine the combinations carried in the drainage waters. This information as to magnesium calcium outgo is of value in considering reasons for the occurrence and subsequent disappearance of the toxicity found in the nearly parallel cylinder experiments. MacIntire, Willis and Holding (24) and subsequent unpublished data have shown the tendencies of the oxides and carbonates of calcium and magnesium to materially alter the normal oxidative processes responsible for the generation of leached sulfates. Table 9 shows the ratio of the total nitrates and sulfates to the amount of bicarbonates leached. The totals of magnesium and calcium carried through as neutral salts, expressed as calcium-carbonate-equivalence, are used in comparisons with the total magnesium and calcium bicarbonates, determined by titration and expressed in terms of calcium carbonate. Direct determinations of potassium in the leachings for the initial 5-year period previously reported by the senior author (20) together with differential determinations of concurrent sodium have established the fact that these alkalies did not come out in large enough quantities to alter the general relations. Table 9 shows that the clay subsoil consistently stopped both neutral salts and bicarbonates of both calcium and magnesium. It may be assumed that the upper part of the subsoil became

TABLE 9

Amounts of magnesium and calcium salts from 8-ton equivalent treatments of 93 per cent CaO, MgO, $CaCO_3$ and $MgCO_3$, per 2,000,000 lbs. of soil in terms of $CaCO_3$ equivalence as lbs. per acre—Annual periods 1914–1919

TANK NUMBER	TREATMENT	AS TO SUBSOIL	1914–1915			1915–1916			1916–1917			1917–1918			1918–1919			TOTALS 1914–1919		
			Total calcic and magnesic salts as $CaCO_3$	Calcium and magnesium bicarbonate		Total calcic and magnesic salts as $CaCO_3$	Calcium and magnesium bicarbonate		Total calcic and magnesic salts as $CaCO_3$	Calcium and magnesium bicarbonate		Total calcic and magnesic salts as $CaCO_3$	Calcium and magnesium bicarbonate		Total calcic and magnesic salts as $CaCO_3$	Calcium and magnesium bicarbonate		Total calcic and magnesic salts as $CaCO_3$	Calcium and magnesium bicarbonate	
				As $CaCO_3$	As per cent of total salts		As $CaCO_3$	As per cent of total salts		As $CaCO_3$	As per cent of total salts		As $CaCO_3$	As per cent of total salts		As $CaCO_3$	As per cent of total salts		As $CaCO_3$	As per cent of total salts
			lbs.	*lbs.*	*per cent*	*lbs.*	*lbs.*	*per cent*	*lbs.*	*lbs.*	*per cent*	*lbs.*	*lbs.*	*per cent*	*lbs.*	*lbs.*	*per cent*	*lbs.*	*lbs.*	*per cent*
1	CaO	None	1879	494.9	26.3	1123	665.8	59.3	1045	750.6	71.8	485	330.7	68.2	776	449.7	57.5	5308	2691.7	50.7
22	CaO	1 foot	778	112.4	14.4	708	176.4	24.9	733	203.9	27.8	362	148.8	41.1	565	154.3	27.8	3146	795.8	25.3
2	MgO	None	2454	1128.7	46.0	1420	1032.8	72.7	1612	1150.7	71.4	631	469.6	74.3	940	586.4	62.4	7057	4368.2	61.9
23	MgO	1 foot	678	108.0	15.9	838	168.6	20.1	816	231.5	28.4	447	197.3	44.1	546	180.8	33.1	3325	886.2	26.7
3	$CaCO_3$	None	1555	181.9	11.7	889	478.4	53.8	987	622.8	63.1	501	316.3	63.1	797	476.2	59.8	4729	2075.6	43.9
24	$CaCO_3$	1 foot	534	115.7	21.7	600	154.3	25.7	791	217.1	27.4	379*	43.0*	11.3	684	187.4	27.4	2988	717.5	24.0
4	$MgCO_3$	None	3714	2432.7	65.5	1870	1494.7	79.9	1714	1369.0	79.9	687	504.8	73.5	900	563.2	62.6	8885	6364.4	71.6
25	$MgCO_3$	1 foot	598	108.0	18.0	808	179.7	22.2	1031	249.1	24.2	411	164.2	40.0	573	181.9	33.5	3421	882.9	25.8

* Outlet became plugged which produced waterlogged condition and reduced number of leachings until trouble was rectified.

saturated in effecting this absorption phenomenon and that the plant roots had access to the calcium and magnesium held in this way. Except for this point, the data relating to the tanks with subsoil will not be considered further. The CaO and $CaCO_3$ additions did not prove toxic when applied in nearly equal amounts in the cylinders and are included only because of the comparative relationships. The data on the chemically equivalent application of magnesium oxide are of value because of relationship to the carbonate and for some references yet to be made.

Bicarbonates, known by analysis to be principally magnesium bicarbonate, leached from cylinders treated with magnesium oxide and carbonate amounted, respectively, to 46.0 per cent and 65.5 per cent of the total alkali-earth losses during the initial year. These magnesium bicarbonate losses from the magnesic additions are materially greater than those of calcium bicarbonate from the corresponding treatments of calcium oxide and carbonate. The total outgo of alkali-earths decreased after the initial year. The same is true of both neutral salts and bicarbonates; but, the greater relative decrease in the former causes a general increase in the percentage relationship of bicarbonates to the total, this being true for both calcium and magnesium additions. However, table 13 shows that the increasing percentage of bicarbonates to the total is derived mainly from residual calcium carbonate in the case of the calcium treatment; while a lesser carbonate residual in the case of additions of magnesium oxide and carbonate means a greater supply of magnesium derived through the hydrolysis of the absorbed non-carbonate residuals from the added magnesic materials. In spite of the large residual stores of magnesic materials, it is evident that the amounts of magnesium carried by the leachings are materially decreased after the first year. In the case of the MgO treatment, it appears that the decrease is mainly due to a lessened production of neutral salts during the second and third year more particularly, since the absolute amount of bicarbonates yielded each year of this two-year period closely approach the outgo during the initial annual period. However, in the case of the magnesium carbonate treatment, both neutral magnesium salts and bicarbonates suffer a marked decrease, the ratio of bicarbonate to total magnesium salts increasing somewhat during both the second and third yearly periods. The decrease during the fourth and fifth annual periods is still more marked, notwithstanding the magnitude of the differentials between the applied materials and the recoveries. Such differentials represent well disseminated residuals which would be expected to yield quantities ample to effect an approximation to the concentrations maintained by the earlier leachings, if the remaining stores were readily soluble. It would thus appear that the large amount of magnesium remaining had become less soluble. For the 5-year period, 1914–1919, comparable to the 5-year period, 1913–1918, in the cylinder experiments, the total magnesium outgo from the MgO treatment was equivalent to 7057 pounds of calcium carbonate. Of this amount an outgo of 4368 pounds, or 61.9 per cent, is accounted for by the bicarbonates of the

alkali-earths, as against 2689 pounds, or 38.1 per cent, for the neutral salts of the same elements. In the case of the corresponding $MgCO_3$ addition, the total magnesium outgo equivalent to 8885 pounds of $CaCO_3$ is accounted for by assigning 6362 pounds, or 71.6 per cent, to the bicarbonate form and 2523 pounds, or 28.4 per cent, to the neutral salts.

SOLUBILITY OF THE FIXED MAGNESIUM DERIVED FROM THE MAGNESIUM CARBONATE TREATMENTS—CYLINDER EXPERIMENTS

The ready solubility of the large non-carbonate magnesium residual derived from the applied carbonate shortly after its incorporation may be reasonably assigned as the cause of the initial toxicity. A diminished solubility 5 years later may be attributed to decreased solubility of the residual, or a marked decrease in the amount of the residual. Were the magnesium combinations of the soil of the same chemical composition at the time of the first and fifth year's samplings, the comparative solubilities would be determined by the total amounts remaining at the two dates and upon variation in the tenacity of the fixation, or complexity, as influenced by the time factor, that is, the aging process. Data upon this point were secured by extractions with carbonated water, with a solution of ammonium carbonate and also with hydrochloric acid.

TABLE 10

Calcium and magnesium dissolved by a 4-hour agitation and extraction with carbonated water from 25-gm. charges of composite samples from rims treated separately with precipitated $CaCO_3$ and precipitated $MgCO_3$, June, 1913

TREATMENT		DATE OF SAMPLING	TOTAL CHEMICAL EQUIVALENCE OF TITRATIONS OF CARBONATED WATER EXTRACT		CALCIUM		MAGNESIUM	
Material applied to soil	$CaCO_3$ or $\Rightarrow$ per 2,000,000 lbs. of soil		As $CaCO_3 \Rightarrow$	As $CaCO_3$ per 2,000,000 lbs. of soil	As $CaCO_3$ per 25 gm. of soil	As $CaCO_3$ per 2,000,000 lbs. of soil	As $\Rightarrow CaCO_3$ per 25 gm. of soil	As $\Rightarrow CaCO_3$ per 2,000,000 lbs. of soil
	lbs.		*gm.*	*lbs.*	*gm.*	*lbs.*	*gm.*	*lbs.*
None*		May 6, 1918	0.0250	2000	0.0080	640	0.0128	1024
$MgCO_3$†	28180	Aug. 15, 1913	0.0535	4280	0.0083	664	0.0580	4640
$MgCO_3$†	28180	May 6, 1918	0.0380	3040	0.0066	528	0.0300	2400
$CaCO_3$†	28180	June 18, 1918	0.2420	19360	0.2355	18840	0.0082	656

* Composite of 4 cylinders.

† Composite of 8 cylinders.

Solubility of residual magnesium in carbonated water

In determining the solubility of calcium and magnesium in the soil of the magnesium-treatment cylinders, the sampling of August 15, 1913 was run in parallel with that of May, 1918. The calcium carbonate treatment cylinders

and the control, both sampled in 1918, were also extracted in parallel. Composite samples were made by taking equal amounts from each of 8 magnesium carbonate treatments of both sampling dates, while the same number of subsamples was used in a composite sample of the calcium carbonate treatments. One control composite was made by the use of four no-treatment cylinders and another by the use of eight. A 25-gm. charge of each composite was placed in a 500-cc. cylinder and subjected to agitation by a rapid current of purified CO_2, and by frequent shaking. The solutions thus secured after four hours contact were immediately filtered through a 10-cm. Büchner filter, and titrated with 0.05 *N* acid, using methyl orange as an indicator. The titration values and also the determinations of calcium and magnesium thus obtained are given in table 10. It will be noted that the recovery of calcium, as the bicarbonate from the calcium carbonate treatment of the 1918 sampling, was very much greater than that of magnesium from the magnesium carbonate treatment as sampled in 1913, 8 weeks after the incorporation of the treatment. This is the reverse of what would have obtained had both calcium and magnesium carbonates been respectively present in excess. The corresponding disparity between the calcium dissolved from the calcium carbonate treatment of the 1918 sampling and that of magnesium from the 1918 sampling of the magnesium carbonate treatment cylinders was still greater. The calcium carbonate treatment residual was present, however, mainly as the carbonate, while there was no carbonate residual from the magnesium carbonate additions at the time of either the 1913 or the 1918 sampling. The natural leaching to which the cylinders were exposed under humid conditions precluded the accumulation of any appreciable amounts of soluble calcium and magnesium salts prior to the rapid conversion to the air-dry condition. Hence, the solvent action of the carbonated water was exerted in the main upon the residual unabsorbed excess of the precipitated calcium carbonate, in the case of the soils from the cylinders treated with this carbonate; while, in the case of the magnesium treatment, the solvent action was exerted largely as a result of the hydrolysis of the less soluble silicates. There was no great difference between the amounts of calcium dissolved from the untreated soil and that from the two cylinders which received the treatment of magnesium carbonate; however, there was found an appreciable difference between the total titration values and also the occurrences of magnesium. Both the total titration value and the amount of magnesium of the first, or 1913, sampling were greater than the corresponding findings for the second, or 1918, sampling. The amount of magnesium extracted from the precipitated calcium carbonate treated soil was considerably less than that dissolved from the control. This would indicate either one or both of two possibilities—the residual magnesic combinations may have been decreased in the case of the calcium carbonate treatment as the result of an earlier substitution of calcium for magnesium in the siliceous complexes native to the soil; or, the solvent action of the calcium-carbonate-charged carbonated water was less active than the water,

TABLE 11

Calcium and magnesium dissolved by simultaneous 4-hour agitation and digestion of 25-gm. charges of composite samples from cylinders treated separately with precipitated $CaCO_3$ and precipitated $MgCO_3$ by a 10 per cent $(NH_4)_2CO_3$ solution—controls of precipitated $CaCO_3$, precipitated $MgCO_3$ and serpentine in parallel. Treatments applied June 13, 1913 at rate of 28,180 pounds $CaCO_3$, or equivalent, per 2,000,000 pounds of soil

MATERIAL APPLIED TO SOIL	DATE OF SAMPLING	CONTROL MATERIAL	CALCIUM			MAGNESIUM		
			As $CaCO_3$ per 25 gm. of soil	As $CaCO_3$ in soil	As $CaCO_3$ per 2,000,000 lbs. of soil	As $CaCO_3$ ⇔ per 25 gm. of soil	As $CaCO_3$ ⇔ in soil	As $CaCO_3$ ⇔ per 2,000,000 lbs. of soil
			gm.	*per cent*	*lbs.*	*gm.*	*per cent*	*lbs.*
None*	June 18, 1918		0.0003	0.0012	24	0.0046	0.0184	368
Ppted. $MgCO_3$*	Aug. 15, 1913		0.0003	0.0012	24	0.0895	0.3580	7160
Ppted. $MgCO_3$*	May 6, 1918		0.0029	0.0116	232	0.0512	0.2048	4096
Ppted. $CaCO_3$*	June 18, 1918		0.0003	0.0012	24	0.0131	0.0524	1048
		0.2 gm. $MgCO_3$†	0.0027	0.0108	216	0.1913	0.7652	15304
		0.2 gm. $CaCO_3$	0.0003	0.0012	24	0.0000	0.0000	0
		0.25 gm. serpentine	0.0000	0.0000	0	0.0082	0.0328	656

* Composite of 8 cylinders.

† Completely dissolved.

relatively free of the calcic salt, upon the magnesium silicates and possibly other complexes. The silicates formed as a result of the absorbed additions may be considered, in one sense, as of the same age in the two samplings. However, the samples taken in 1913 were immediately air dried and remained in that condition during the interval of nearly 5 years, during which time the soil of the 1918 sampling was continually under moist exposure and the dynamic activities of the soil, though it is of course true that the latter sampling had been subjected also to a decreased magnesium content through the process of leaching during the same subsequent period above specified. However, it is indicated quite conclusively that the plants growing in the soil from the magnesium carbonate cylinders were subject most probably to the toxic influence of a larger amount of magnesium, both as bicarbonate and as neutral salts, during the growing season of 1914, than was the case while the plants were growing in the season 1918.

Solubility of residual magnesium in a solution of ammonium carbonate

The solubility of the magnesium non-carbonate residuals from the absorbed carbonate treatments was determined by a 4-hour digestion with a 10 per cent aqueous solution of ammonium carbonate, the results of which are given in table 11. The sampling of August 1913 and a corresponding sampling of May 1918 were used, each charge being from a composite of the sampling of 8 cylinders. The precipitated calcium carbonate treatment and the control, both sampled in June, 1918, were used as parallels. Additional controls of 0.20 gm. of precipitated $MgCO_3$ and the same charge of precipitated $CaCO_3$ were included, together with a 0.25-gm. charge of finely ground serpentine which had been boiled with dilute hydrochloric acid for one minute in order to remove any trace of carbonate. For convenience of comparison, the calcium and magnesium determinations from these controls were calculated to the soil basis, as though the soil and controls were included in the same charge. It will be noted that the entire 0.20-gm. charge of precipitated magnesium carbonate was dissolved by the ammoniacal carbonate solution. This charge was equivalent to more than double the maximum amount of carbonate dissolved from the soil charges. The amounts of magnesium dissolved by the ammonium carbonate solution were about twice as great as the corresponding extractions by carbonated water, save in the case of the no-treatment cylinder composites. The amount of magnesium dissolved from the composite of the samplings of 1913 was about twice as great as that from the corresponding composite of the 1918 samplings, being in this connection in parallel with the extractions with carbonated water. The ammoniacal carbonate solution was, however, practically inert toward the occurrence of an excess of residual calcium carbonate and upon the control charge of the same alkali-earth carbonate. The fact that more magnesium was extracted from the 1918 composite of the calcium carbonate treatment than from the 1918 sampling of the control may be construed as indicating that the applied calcium carbonate had served to convert

the native magnesium complexes into forms more readily hydrolyzed. The action of ammonium carbonate on the 1913 sampling demonstrates that the non-carbonate residual from the absorbed applied carbonate was still responsive to that solvent. However, the moist condition was maintained for only the initial 8 weeks after the treatment. In the case of the 1918 sampling it is probable that both ageing and leaching over the longer period are controlling factors responsible for the smaller magnesium recovery when simultaneous extractions of the two samplings were made. This relationship is in harmony with that found in the carbonated-water extractions.

TABLE 12

Solubility of residual calcium and of non-carbonate magnesium in composite samples from cylinders treated separately with precipitated $CaCO_3$ and precipitated $MgCO_3$, June 13, 1913,—4 hour boiling digestion with 1-1 HCl, 5-gm. charge

TREATMENT		DATE OF SAMPLING	CALCIUM		MAGNESIUM	
Material applied to soil	$CaCO_3$ ⇔ per 2,000,000 lbs. of soil		As $CaCO_3$ in soil	As $CaCO_3$ per 2,000,000 lbs. of soil	As $CaCO_3$ ⇔ in soil	As $CaCO_3$ ⇔ in 2,000,000 lbs. of soil
	lbs.		*per cent*	*lbs.*	*per cent*	*lbs.*
None	None	June 18, 1918	0.2510	5020	0.7548	15096
Ppted. $MgCO_3$*	28180	Aug. 15, 1913	0.3137	6274	1.0800	21600
Ppted. $MgCO_3$*	28180	May 6, 1918	0.2967	5934	1.0120	20240
Ppted. $CaCO_3$*	28180	June 18, 1918	1.6487	32974	0.7674	15348

* Composite from 8 cylinders.

Solubility of residual magnesium in 1-1 hydrochloric acid

The amounts of magnesium dissolved from the magnesium carbonate treated cylinders by boiling with 1-1 hydrochloric acid, as compared to those dissolved from the calcium carbonate and untreated cylinders, are given in table 12. Five-gram charges of each composite were boiled for 4 hours with 250 cc. of 1-1 hydrochloric acid in 500-cc. Kjeldahl flasks, fitted with funnels to serve as reflux condensers. The solutions were filtered hot, the residues again suspended in dilute hydrochloric acid and washed upon a 10-cc. Büchner with hot distilled water. The filtrates were evaporated and silica dehydrated. The residue was taken up with dilute hydrochloric acid and the iron thrown out in mechanical combination with the dehydrated silica. The hydrated iron oxide residue was twice re-dissolved, re-filtered and re-precipitated. The combined filtrates from the calcium were evaporated with 25 cc. of nitric acid; taken up; made ammoniacal, manganese precipitated by the addition of bromine and filtered, after which magnesium was determined as magnesium pyrophosphate. The amounts of magnesium thus determined in the charges from the untreated cylinders and those from the cylinders which received the precipitated carbonate of calcium were practically the same. The tendency of the heavy additions of calcium carbonate to liberate magnesium from the soil was offset

apparently by the magnesium content of the heavy application of manure which was made in the case of 4 of the 8 cylinders sampled to secure the composite. The 1913 sampling composite from the 8 cylinders, to which the heavy addition of magnesium carbonate had been made, yielded but 1360 lbs. more per 2,000,000 lbs. of soil than was obtained from the corresponding composite sampled almost 5 years later. This difference is interesting as compared to the much larger corresponding differences of 2240 lbs. and 3064 lbs. obtained by the carbonated water and ammonium carbonate extractions, respectively. The comparison indicates that the differential solubility at the two periods of sampling, as indicated by the two less active solvents, is a more potent factor than is the total, or near-total, amount indicated by the solvent action of the more active acid solvent. The three solvents, carbonated water, ammonium carbonate and hydrochloric acid afford recoveries in magnitude corresponding to the given order. Considering the results secured by these three solvents together with the leaching data, it is evident that, with the advent of fertility, the combined magnesium is now held more tenaciously by the cylinder soil which was subjected to exposure and dynamic conditions for the 5-year period than it was by the same soil which remained under such conditions for only a few weeks.

PREVIOUS OBSERVATIONS RELATIVE TO MAGNESIUM-INDUCED TOXICITY AND ITS SUBSEQUENT DISAPPEARANCE

The disappearance of initial toxicity and the advent of fertility, where no assignment of cause can be made to the development of crop tolerance has received little, if any consideration. The problem of the cumulative toxic effect of moderate magnesium additions is distinct from the present consideration of that encountered as the initial effect of single heavy applications. The problem of MgO additions involves factors not involved in $MgCO_3$ additions; while still another problem arises when we apply either dolomite or magnesite.

The reversion of an excess of the oxide to the carbonate in the soil, in time, makes the usage of the oxide comparable to that of the carbonate. Magnesium oxide additions were made in some of the experiments the results of which are reported in part in this contribution. No seedings were made, however, where the oxide was added. However, data were secured as to the respective chemical activities of the two forms after their incorporation with the soil. The problem of toxicity caused by accumulations of magnesium carbonate from heavy additions of magnesium oxide and that caused by direct applications of the carbonate will be considered as related and comparable.

It appears that "burned" magnesian lime has been used for a long period in the British Isles. In discussing observations made by Tenant in 1800, and his own experiments conducted in 1806 and 1810, Sir Humphrey Davy (2) states that "a particular species of limestone found in different parts of the North of England, when applied in its burnt and slaked state to land, in considerable quantities, occasioned sterility, or considerably injured the crops for many years," the toxic quality having been assigned to the occurrence of "mag-

nesian earth." Davy concluded that magnesium oxide was slow to carbonate and that toxicity persisted during the continued presence of the uncarbonated oxide; but, that "magnesia in its mild state, i.e., fully combined with carbonic acid, seems to be always an useful constituent of soils." He made mixtures of soil and peat and observed a large formation of carbonate with the decreased proportion of silicates—synonymous with lessened silicate fixation and decreased magnesium solubility. He advocated the use of peat in practice, in order to effect greater carbonation and eradication of toxicity.

In his critical review upon the validity of the experiments reported upon the influence of magnesium as related to the hypothesis of Loew, Lipman (11) aptly stressed "the lack of extensive field experiments," and also, as more germane to our treatise, "the very short period allowed in the different experiments for testing the hypothesis." Hopkins (8) conducted a 5-year pot study relative to the manurial value of magnesium carbonate applied at 5 rates; and the removal of any harmful excess by the leaching of magnesium sulfate formed from additions of calcium sulfate. He found a distinct tendency toward a reduction in toxicity after the third year, even in the case of the 20-ton unleached $MgCO_3$ treatment without calcium sulfate. This tendency toward improved plant growth in the presence of magnesium accumulations was attributed by Hopkins to the development of a magnesium-resistant strain of wheat. Lipman (11) observed a relation between time and a tendency toward reduced toxicity. He found that an initial toxicity toward citrus trees was mitigated after one and one-half to two years of fallow. Wheeler (34) and Wheeler and Hartwell (35) carried out a field cylinder experiment wherein magnesium oxide was applied, with later supplement of magnesium carbonate. Accounting for an initial toxicity which was followed by a beneficial effect, Wheeler (34) advances the explanation—"The injurious action of the caustic magnesia may, therefore, have been due to the fact that it was too caustic to be endured when in direct contact with the roots of the plants, a condition which was overcome before the succeeding year by virtue of its union with more carbon dioxide," and, "the preceding results show that caustic magnesia was toxic at first when it was used in large quantities, even on a soil evidently in slight need of magnesia, but that when sufficient opportunity had been afforded for it to become carbonated, it became useful." In harmony with our findings relative to the accumulation of neutral magnesium salts from carbonate additions and absence of lethal toxicity, Wheeler and Hartwell found no detrimental effects when neutral magnesium chloride was applied to the soil of the cylinders. Loew (14) contends, however, that the persistence of the magnesium oxide—when magnesium is applied in that form—because of the less rapid reversion to the carbonate, is not the true reason for the toxicity attributed to certain New Jersey limestones.

THE TOXICITY INDUCED BY MgO AND $MgCO_3$

The foregoing citations and quotations are in advocation of the viewpoints; that MgO was more toxic than an equivalence of $MgCO_3$; that an application of MgO might be harmful as such, yet beneficial after its conversion to $MgCO_3$; that such carbonation was not rapid, and that the oxide-derived $MgCO_3$ was considered to be of comparable soil-reactivity with calcium carbonate. A more extensive parallel study of the reversions of CaO to $CaCO_3$ and MgO to $MgCO_3$ will be considered in another paper. However, the action of an 8-ton CaO-equivalence of each of the two oxides and carbonates, in both cylinders and lysimeters will be considered here, insofar as the parallel may be directly germane.

Frear (4) treated several soils with CaO in parallel with MgO and determined the increase in carbonate accumulations after a period of 10 months. The increase in carbonate was found to have been greater in the case of the CaO

additions than where MgO additions had been made, which led Frear to conclude that the MgO was still, to a large extent, uncarbonated. The intensity of the magnesia-silica reaction had not been pointed out at that time and it is probable that the low carbonate results from the MgO treatments were due in part, at least, to the ready reaction between soil and that part of the MgO which had been converted to magnesium carbonate. Frear wrote later in his classical compilation (5): "In view of MacIntire's results, it is doubtful whether this basic carbonate could accumulate in quantities sufficient to be influential." Schollenberger (30) has offered, more recently, a contribution to this phase of the subject.

Our cylinder results have demonstrated the presence of toxicity from additions of magnesium carbonate, rather than magnesium oxide; and the further fact that the initial toxicity was manifested after the decomposition of the carbonate and the fixation of the magnesium by the soil. The lysimeter studies with the same soil and the same amounts of $MgCO_3$, and also equivalences of MgO, CaO and $CaCO_3$, throw additional light upon the fixation and leaching of the carbonates, both applied and engendered, and the speed of the reversion, to the carbonate. The data of table 13 bear upon the latter point.

The calcic and magnesic additions were mixed throughout the entire amount of soil in the respective tanks. Carbonate-CO_2 determinations were made seven times during the first year and at the end of the second and third annual periods. An unsupported comparison between the determination of residual carbonates derived from chemically equivalent treatments of CaO and MgO at the 8-ton CaO rate would indicate that the CaO had reverted to the carbonate form much more rapidly than had the MgO. The highest $CaCO_3$ occurrences from the calcium oxide amounted to .7783 per cent; while the highest $CaCO_3$ equivalence from MgO, after the first 10 day interval, amounted to but .1050 per cent in the surface-soil-only series. In like manner, corresponding figures of .8034 per cent and .1143 per cent were the maxima obtained after 17 days in the series which included subsoil beneath the treated surface soil. Without the inclusion of the respective control treatments of equivalent carbonates to establish the parallel as to their respective reactions with the soil, these data would undoubtedly lead to the same conclusions as those drawn by Wheeler and by Frear. However, when the residual carbonates from the precipitated carbonate treatments are included in the comparison, there arises an entirely different deduction, relative to the speed of the respective carbonations of CaO and MgO. An initial carbonate residual of approximately 0.9 per cent for both series is found as a result of the $CaCO_3$ additions, as against around 0.3 per cent as an average of the two $MgCO_3$ additions, after moist soil and the carbonates had been in contact for 10 days without leaching. The two calcium carbonate treatments decreased slowly in their residual carbonate-CO_2, until an occurrence of 0.68 per cent $CaCO_3$ was found, in both cases, at the end of three years. On the other hand, the magnesium carbonate additions

TABLE 13

Residual carbonates in terms of $CaCO_3$ per acre of 2,000,000 lbs. of soil from analyses of lysimeter soils treated separately with CaO, MgO, $CaCO_3$ and $MgCO_3$ at the equivalent rate of 8 tons of CaO per acre 2,000,000 lbs. of soil. Analyses were made on moist soil but are reported on moisture free basis.

DATE OF SAMPLING	TIME BETWEEN TREATMENT AND SAMPLING	SURFACE SOIL ONLY								SURFACE SOIL OVER SUBSOIL							
		CaO		MgO		$CaCO_3$		$MgCO_3$		CaO		MgO		$CaCO_3$		$MgCO_3$	
	days	*per cent*	*lbs.*	*per cent*	*lbs.*	*per cent*	*lbs.*	*per cent*	*lbs.*	*per cent*	*lbs.*	*per cent*	*lbs.*	*per cent*	*lbs.*	*per cent*	*lbs.*
7–27–14	10	.....	.....	0.1657	3314	0.8935	17970	0.2939	5878	0.5493	10986	0.1034	2068	0.8852	17704	0.3609	7218
8– 3–14	17	0.4888	9776	0.0818	1636	0.8955	17910	0.2716	5432	0.5811	11622	0.1207	2414	0.8928	17858	0.3530	7060
8–18–14	32	0.5763	11526	0.1050	2100	0.9115	18230	0.1838	3672	0.6116	12232	0.1016	2032	0.8966	17932	0.2184	4368
9– 4–14	49	0.6657	13314	0.0795	1590	0.8350	16700	0.1483	2966	0.6584	13168	0.0459	918	0.8779	17558	0.1975	3950
9–29–14	74	0.7252	14504	0.0625	1250	0.9343	18686	0.1675	3350	0.8034	16068	0.1143	2286	0.9597	19194	0.2461	4922
2–20–15	218	0.7532	15064	0.0761	1522	0.8877	17754	0.1293	2586	0.8007	16014	0.0725	1450	0.8704	17408	0.1002	2004
6–18–15	360	0.7783	15568	0.1004	2008	0.8388	16776	0.0994	1988	0.7588	15176	0.0823	1646	0.8341	16682	0.1090	2180
7– 5–16	720	0.6974	13948	0.0253	506	0.7508	15016	0.0228	456	0.6804	13608	0.0363	726	0.7471	14942	0.0332	664
7–15–17	1080	0.6410	12820	0.0055*	110*	0.6880	13760	0.0060*	120*	0.6040	12080	0.0105*	210*	0.6830	13660	0.0080*	160*

* Probable error in the analysis of a 20-gm. charge amounted to 0.0068 per cent, or 136 pounds.

gave insignificant carbonate occurrences at the end of the same period, the small traces found being within analytical error. The differences in gains of caronate-CO_2 could not be accounted for by the greater solubility of the engendered $MgCO_3$, as compared to that of the $CaCO_3$ formed, and the greater consequential leaching of $MgCO_3$; for, the total magnesium leached, both as neutral salts and as bicarbonates, amounted to but 5486 pounds from the MgO added to the surface soil. Of this amount, only 3312 pounds came through as bicarbonates; while, of the total leaching of all forms, amounting to 7298 pounds, in the parallel application of $MgCO_3$, only 5296 pounds were to be accounted for by the presence of bicarbonates. The average of the nine determinations fo $CaCO_3$-equivalent residual from the precipitated calcium carbonate was 16978 pounds during the first three years, in the case of the surface-soil-only series, as against 2938 pounds for the magnesium carbonate. On the other hand, corresponding values of 16993 pounds and 3613 pounds, respectively, were obtained from the soil treatments in the subsoil series. It is apparent, therefore, that the large carbonate residual derived from the added CaO accumulated not alone because of more rapid carbonation, but because of the additional fact that the calcium carbonate was not so quickly, nor so extensively, fixed by the soil. The analyses of the leachings from the lysimeters showed that the magnesium treatments caused a somewhat larger outgo of nitrates and sulfates and this factor and that of the greater solubility of the magnesium carbonate account for part of the disparity between the residuals of the carbonates of magnesium and calcium.

It is true that MgO hydrates less readily than does CaO under exposure to normal atmospheric conditions, and the subsequent conversion to the carbonate is, therefore, less speedy because of this less rapid conversion to the essential preliminary combination under the stated conditions. However, in suspension of the two oxides in carbonated water at ordinary laboratory temperature, where the supply of CO_2 is maintained by steady influx at atmospheric pressure, the amount of magnesium carbonate formed and ultimately dissolved as bicarbonate, is approximately thirty times as great as that of calcium bicarbonate. Such a condition would be approached more nearly by the soil moisture than through ordinary exposure to the atmosphere. Thus, particularly in the case of heavy additions, we would expect to find that while the magnesium oxide applied to the soil might be reverted to the carbonate more slowly than would lime, as a result of less rapid hydration essentially precedent to carbonation, the soil solution would soon become more heavily impregnated with magnesium bicarbonate than with calcium bicarbonate. The leaching data proved the correctness of this assumption.

Because of the intimate mixture of soil with oxide, hydroxide, interspersed granules of carbonate and freshly formed silicates in the lysimeters, it would be difficult to assign definite approximate values to the several magnesic combinations as sources of the leached magnesium. Variations in the amounts of CO_2 liberated from the carbonate form, and thus made available for either

the further carbonation of the uncarbonated hydroxide, or for the more extensive hydrolysis of the magnesic siliceous complexes would, of course, materially affect both the total amount and the form of the leached magnesium. Considering the ready solubility of the pulverulent carbonate, and the strong tendency of this compound to be fixed by the soil, together with the ready conversion of the hydroxide—always a constituent of the basic carbonate—it would seem logical to conclude somewhat as follows. The $MgCO_3$ would react with the acid silicates and with biologically engendered acids, causing a liberation of CO_2. This would be taken up in large part by the $Mg(OH)_2$, with the further fixation of $MgCO_3$, or leaching of the resultant bicarbonate. After the conversion of the hydroxide to the carbonate, there would still be dynamic activities wherein CO_2, liberated by decomposition of absorbed magnesium carbonate would then tend to form carbonated water, which would be effective in causing the reversal of the fixation process through hydrolysis and carbonation of the hydrolyzed derivative from the silicate. But, as indicated by the leaching data, the magnitude of the products of such dynamic conditions and the tendency toward electrolytic dissociation both approach the minimum with increase of time.

THE BEARING OF TRANSIENT TOXICITY UPON LIME-MAGNESIA RATIO STUDIES

The pronouncement by Loew (12) of his optimum lime-magnesia ratio hypothesis and his subsequent ardent advocacy of his viewpoint inspired a number of investigations upon the subject. The biological phase of the problem and the possible antagonistic relationships between calcium and magnesium have been also extensively studied. A number of studies have been carried out in water cultures, to the exclusion of the vitiating influence of the solid phase, soil mass or quartz sand. The late Dr. Wm. Frear (5) very aptly drew attention to the fallacy of applying results obtained in water cultures to the practice of liming with calcareous and dolomitic products, when he wrote—"From the fact of water culture experiments with calcium and magnesium chlorides, nitrates and sulfates, to those of the influence of calcareous or magnesium dressings, as oxides, hydrates or carbonates upon cropped land, is a very long road with many streams to cross." Through citations, McCool (25), also, has stressed the function of the solid media in ameliorating the tendencies exerted by the soluble alkali and alkali-earth salts. Such water culture experiments are not considered by us as being closely related to the soil studies offered in this contribution.

The findings reported in this paper were not obtained in an effort to determine the persistency of magnesia-induced toxicity, nor in a study of the Loew hypothesis, as such. It is not intended, therefore, to offer an extensive survey of the lime-magnesia investigations. Such has been done in the bibliographies compiled by Loew (13, 14), Lipman (11), Gile (6) and McCool (25). However, the results relative to the differential extent of $CaCO_3$ and $MgCO_3$ fixations, and the differences between CaO and MgO transformations into carbonates in the atmosphere as compared with such transformations in the body of the soil were not known when many of the earlier experiments were carried out. Furthermore, the studies were usually of such brief duration that it had not been observed that an initial toxicity might be converted later into a beneficial effect, with but little difference in the amounts of magnesium present under the two conditions. These later findings introduce factors which were not taken into account in many of the earlier related investigations.

In those instances where the equivalent alkali-earth carbonate additions were in such small amounts that all of both calcium and magnesium carbonates were fixed, the variations between

the solubilities of the fixed derivatives may not have been of great import. But, when the additions were heavy and of chemical equivalence, the unabsorbed $CaCO_3$ residuals may have been compared with magnesium silicate complexes, rather than a residual of magnesium carbonate. In such cases, the original comparative solubilities of the two carbonates do not apply, but rather a new and reverse order of solubility. Again, it has been shown (19, 23) that MgO will quickly revert to the carbonate in suspensions of carbonated water. However, with less opportunity for diffusion of the weaker carbonated solution, the magnesium carbonate will be formed not so readily in the soil and it is there subjected to both leaching and fixation. However, this chemical parallel is not complete in the case of sand cultures. Hence the plant growth results from CaO-MgO comparisons in sand culture experiments may not be properly considered as having unqualified application to soils, even with the exclusion of biological influences. Again, we would stress that unfortunately many of the previous experiments were of but brief duration, affording no opportunity to establish the fact that the initial effects may not be necessarily permanent.

Loew (13, 14) pointed out that the variation in the solubilities of the several solid-phase carbonates, as shown by Ulbricht, was to be considered as an *a priori* factor in ratio studies, drawing particular attention to the difference between the pulverulent precipitated basic carbonate and pulverized magnesite. He also contended that the toxic influence of the basic precipitated carbonate is due to the presence of the hydrate which, however, he maintained would soon be converted to the carbonate. Daikuhara (1) took cognizance of the disparity between the intensity of the influences exerted by the soluble sulfate of magnesium and that exerted by the precipitated carbonate of the same element in antagonistic action upon precipitated calcium carbonate present in sand cultures, wherein rice was grown. Nakamura, (28) in studying magnesium deficiency, makes an interesting observation to the effect that, "the proper way to correct the unfavorable ratio of lime and magnesia of this soil, for the growth of Grammineae, would be to add so much magnesia in the form of insoluble hydrous silicate, or powdered magnesite that the ratio of lime to magnesia becomes 1:1, for the availability of the magnesia would then agree or nearly so with that of the lime."

Our viewpoint, suggested by the data given, is that the silication of the large amounts of magnesium carbonate, which some soils can fix, represents an approach toward the native aged mineral silicates. Nakamura further differentiated between the soluble sulfate and powdered magnesite giving an "agronomical ratio" of 23:100 and he pointed out that an addition of the soluble sulfate should be very much less for a sandy than for a clay soil. Loew and Aso (15) likewise maintained that the proper conception of optimum lime-magnesia ratio is based upon the "condition that *the availability is equal*," the context indicating that the primary consideration entertained by them was the solubility of the applied material, *per se*. However, in discussing the importance of availability coefficient, which they designate as "agronomical equivalent," they do state that "this magnitude changes with the nature of the soils and the partial transformation of the applied compounds into other forms in the soil". Meyer (26) concluded that the deleterious effect brought about by repetitions of magnesia additions were the result of the strong alkaline reaction induced by the added oxide. The hydrated oxide of magnesia is, however, exceedingly insoluble, as compared with hydrated lime and only a small amount of the strongly dissociated hydroxide would be present in the free soil-water, so long as the caustic form persists. But, after reversion of MgO to $MgCO_3$, the magnesium is in a form about 30 times as soluble as that of the corresponding physical form of calcium. Meyer (26) found that magnesia of burned dolomitic limestone and marl to be less harmful than the magnesium oxide derived from calcined magnesite. He concluded that the practical use of dolomitic lime is dependent upon soil's texture and its store of acid-reacting materials, the heavier and more acid a soil, the greater the permissible application of the CaO-MgO, while its use on a sandy soil is deemed not advisable.

As previously stated, it was pointed out by Loew that the pulverulent precipitated magnesium carbonate, which invariably carried some magnesium hydrate, is many times more soluble and consequently more toxic than the native magnesite when applied to soils; but, it

has not been generally accepted that the same variation between the solubilities of precipitated calcium carbonate and finely ground limestone is of sufficient magnitude to be accorded serious consideration. It has been shown, however, by Mooers and MacIntire (27) that the precipitated calcium carbonate is more active than a chemical equivalence of a practical limestone mixture in causing a loss of soluble nitrogen from the soil used in the cylinder experiments reported in this paper.

In a large number of pot studies the calcium and magnesium additions have been in the form of precipitated carbonates, the oxides being used less frequently. It seems to have been tacitly assumed, as a rule, that the chemical activities of the directly applied, or oxide-derived, carbonates of the two elements were of equivalent, or approximate, chemical activity, with a maintenance of their respective solubilities after incorporation with the soil. Again, in the case of heavy applications of precipitated $CaCO_3$ and $MgCO_3$ in chemical equivalence, it was tacitly assumed that both the initial disintegration and the residual occurrences of the two carbonates were in exact, or near, chemical equivalence. This assumption was also proved to be erroneous by the writer and associates (19; 23) who found a marked difference between the speed of the lime-silica reaction and that involving the union of magnesia with silica, as well as an inverse relationship in the speed of the reversal of the reactions through hydrolysis. Little, if any control has been applied to either the differential extent or speed of the reactions responsible for the carbonation, absorption, or fixation phenomena and the variation in the characteristics of the precipitated end-products. In a word, most of the pot and cylinder investigations were carried out without periodic determinations of the disparity between carbonate residuals from the applications of the oxides and carbonates of calcium and magnesium. Neither was much attention paid to the differential tendencies of the absorption end-products to undergo reversal of form, when in contact with carbonated water carrying soil nutrients. The problem has been viewed generally from the angle of the relative effect produced by the two elements through the medium of the applied basic materials, *per se*, or their salts representing biological end-products, rather than those effects induced by their ultimate and more stable combinations in the soil. Little attention was paid to either the distinct variation in the speed of the reactions incident to fixation, or the decided differences in the respective complexities of the engendered silicates of the two alkali-earth bases. Nevertheless, these factors are responsible for chemically characteristic variations in their respective tendencies toward hydrolysis, the initial step in the reversal of the reaction by which, in the absence of the carbonates in the solid phase, the soil solution becomes impregnated with calcium and magnesium bicarbonates. Frear (5), however, apparently had in mind a concept in harmony with the viewpoint which we believe our findings justify, relative to ratio studies, when he wrote: "Either calcium or magnesium brought to the soil in a given state of combination will probably undergo a change of combination after it has been exposed to the soil, and some of it may remain in solution, while another portion is converted to an insoluble compound."

Some of the findings reported in this contribution have a very definite bearing upon the validity of the hypothesis of an approximate optimum lime-magnesia ratio. Loew (13) contends that, "it is always an unfavorable condition when the magnesium content of a soil is essentially higher than the lime content." The results of tables 8, 10, 11 and 12 do not sustain such a contention. Table 8 shows that a large proportion of the added magnesium carbonate is still present, though not in carbonate form, after 5 years. Table 12 shows the residual magnesium to be extensively soluble in hydrochloric acid. Table 11 demonstrates the ready and preponderating solubility of the non-carbonate magnesium residuals in solutions of ammonium carbonate.

But, special stress may be placed upon the data of table 10, since the solubility of the remaining calcium carbonate and that of the absorbed magnesium

residuals in carbonated water is the nearest approach we have to the solvent action of the free soil water which brings supplies of calcium and magnesium to the plant roots. The soil which received precipitated calcium carbonate had become much more productive than the untreated soil at the time of the 1918 sampling. But, with increased productivity, there had come a marked change in the carbonated-water soluble lime-magnesia ratio, an increase from that of 1 to 1.6 to that of 28.7 to 1. Conversely, the magnesium additions had produced definite fertility in 1918, yet they had altered the carbonated-water soluble lime-magnesia ratio from that of 1 to 1.6 to that of 1 to 4.5. Thus, the soil of an original carbonated-water-soluble lime-magnesia ratio of 1 to 1.6 was greatly benefited both by the change to a ratio of 28.7 to 1, and by that of an alteration from 1 to 1.6 to 1 to 4.5. The magnesium additions materially altered the original ratio and produced an excess of both total and available magnesium, the condition termed "unfavorable" by Loew, yet improved growth resulted from the alteration.

In studying lime-magnesia relationships by means of pot or basket experiments, it should be realized that sand (silica) and soil (silicate complexes) are distinctly different media. The two materials vary widely in their abilities to offset the effect of the variation in the active mass resultant from the differential solubilities of the precipitated carbonates of calcium and magnesium in both pure and carbonated water. The silicate complexes are much more active than silica in bringing about an extensive precipitation and fixation or "locking up" of magnesium.

It is readily seen that a moderate or heavy treatment of precipitated $CaCO_3$ or $MgCO_3$ in sand culture would remain largely in the carbonate form, a form somewhat soluble; while in the case of a soil medium the incorporated bases would enter, with distinctly varying degree, into silicate combinations of varying complexicity—forms not so readily soluble in the case of absorbed magnesia. It is, of course, recognized that the simple calcium silicate, $Ca_2Si_2O_6$, is readily hydrolyzed, assuming the improbable formation of so simple a compound, rather than the more probable complex silicates, when lime is fixed by the soil. The texture of the soil is, of course, a factor in the speed of the silication process. The finer separates run parallel quantitatively with greater complexity of silicates, and this tends toward more intensive, speedy, and involved fixation of alkali-earth bases than would be the case with the relatively pure silica of the quartz sand medium. Hence, we find insufficient stress laid on the inherent differences between pot or basket work with sand, a *comparatively* inert medium *at ordinary temperatures*, and similar work with soils carrying varying amounts of the more active acid clay, in large measure finely divided silicates. Without doubt, it is hardly permissible to use the water culture results relative to calcium and magnesium salt relationships, as having unqualified application to plant growth in soils, and we believe the same to be true also in the case of results secured from sand cultures. Our conclusion is that cultures in the relatively inactive acid-silicate-free sand

should not be considered as comparable with lime-magnesia ratio studies in soil media where the different silicates show so much more marked tendency toward the rapid decomposition of the applied carbonates, the rapidity and extent of the fixing of magnesium carbonate being much greater than that of the carbonate of calcium It seems logical to assume that somewhat different conclusions and practical applications might have come from some of the earlier investigations had these facts been given consideration in the control and interpretation of those experiments.

SUMMARY

1. Cylinder studies upon tall-oat grass and cowpeas over a period of years are reported. There prevailed during the initial year a toxicity lethal to three seedings of tall oat grass where heavy additions of precipitated magnesium carbonate had been made, though the carbonate additions had been completely decomposed. This initial toxic condition was succeeded by a beneficial effect.

2. No toxic effect was produced upon either tall oat grass or cowpeas by heavy additions of dolomite, which proved to be about as valuable as limestone of similar fineness and chemical equivalence.

3. Repetition of the cylinder work, as to initial toxicity induced by magnesium carbonate additions upon cowpeas, was made by the use of Wagner pots. Initial toxicity to cowpeas was found; but, this had disappeared at the end of the first year in the unleached pots. A large preponderance of magnesium salts over those of calcium was found in the several zones when the soils were leached at the time of harvesting of the cowpeas, at which time there was no indication of toxicity.

4. Samplings from the cylinders were made in 1913 and 1918 and the pH values of distilled and carbonated water extracts were determined in 1922. The aged magnesic non-carbonate residuals gave values indicating acidity, while the reverse was true in the case of the calcium carbonate residues.

5. Possible reasons for the initial toxicity and its subsequent disappearance were discussed. The data suggested that most probably the aging of the absorbed magnesia and the probable formation of more complex precipitates were responsible for less ready solution in the soil water and for decreased hydroxyl-ion concentrations.

6. Leaching data were given showing the annual and total outgo of magnesium and the progressive decrease in solubility of magnesic additions during the five-year period, in the case of soil used in the cylinders. These additional data established the fact of an occurrence of a large residue of non-carbonate magnesium in the cylinders during the period of lethal toxicity and subsequent fertility. They also determined the forms in which the magnesium was leached from the soil during the prevalence of these two conditions.

7. Information was also obtained relative to the reversible liberative action of excesses of both calcium and magnesium in subsoil, and the effect of the subsoil in stopping the outgo of the two alkali-earth bases.

8. The relative solubility of the cylinder magnesium residuals in the samplings after the first and fifth years was determined by carbonated water, aqueous ammonium carbonate and hydrochloric acid extractions. The large recovery of the unabsorbed $CaCO_3$ and the small recovery of magnesium from the non-carbonate forms in the carbonated water extracts and the variations between samplings are discussed as indicating the influence of the aging of the magnesium complexes. The reverse order of solubility of calcium and magnesium in aqueous ammonium carbonate and the solvent action of HCl are also discussed.

9. The earlier observations relative to toxicity induced by magnesium oxide and precipitated basic magnesium carbonate were considered in the light of the findings relative to the extensive fixation of magnesium by the soil.

10. The parallel between the supposed persistence of applied magnesium oxide and the gradual accumulation of magnesium carbonate, as being responsible for the observed cases of initial toxicity and its subsequent disappearance, were considered in the light of initial toxicity observed by us after the absorption of the applied magnesium carbonate and the later change to a beneficial effect.

11. Residual CO_2 data and leaching results are given showing that the slow hydration and carbonation of MgO in the atmosphere is altogether different from the problem of MgO conversion to carbonate in the body of the soil.

12. The results of the experiments given in this contribution are considered in their bearing upon some of the previous investigations upon the validity of the so-called optimum lime-magnesia ratio hypothesis of Loew. It was pointed out that most of the previous experiments were continued for such brief periods that only the initial effects were observed.

13. Attention is called to the fallacy of using sand cultures as the medium for plant growth, in studies upon the activities of the oxides or precipitated carbonates of calcium and magnesium, when findings so obtained are to be considered as applicable to soils. This, because of the relatively small extent and minimum speed of the reaction between silica and magnesia, as compared to that of the greater extent and speed of the reaction between acid silicates and magnesia.

14. Special stress is laid upon the fact that the comparison of the availabilities of calcic and magnesic additions should not be made upon the basis of the solubilities of the original carriers, but rather upon the solubilities of the absorbed end-products and the unchanged residuals, both of which vary according to the base applied, the form in which they are added, and the period intervening between treatment and analysis.

15. Data are submitted showing that with wide alternating ranges of solubilities of calcium and magnesium, there appeared to be no correlation in the effects produced upon plant growth.

16. It was pointed out that if plant culture experiments relative to the effects of the more active forms of magnesium are conducted over a period of

years, it may be true in many cases, that an initial period of toxicity may be converted into a period of beneficial results—as was found to be the case in our experiments.

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PLATE 1

SHOWING INITIAL TOXICITY INDUCED BY NON-CARBONATE RESIDUES FROM ADDED PRECIPITATED MAGNESIUM CARBONATE AT THE RATE OF 28180 POUNDS $CaCO_3$ EQUIVALENCES PER 2,000,000 POUNDS OF SOIL

Treatments June 11, 1913. Seeding April 7, 1914. Photographed May 27, 1914, just before disappearance of growth on cylinders 31 and 32. Compare with plates 2 and 3.

Treatments per 2,000,000 lbs. soil. Series N

30 No treatment	18 30 tons stable manure	27 28,180 lbs. $CaCO_3$
31 $MgCO_3$ equivalent to 28,180 lbs. $CaCO_3$	32 $MgCO_3$ equivalent to 28,180 lbs. $CaCO_3$ and 30 tons stable manure	21 28,180 lbs. $CaCO_3$ and 48 tons stable manure

PLATE 1

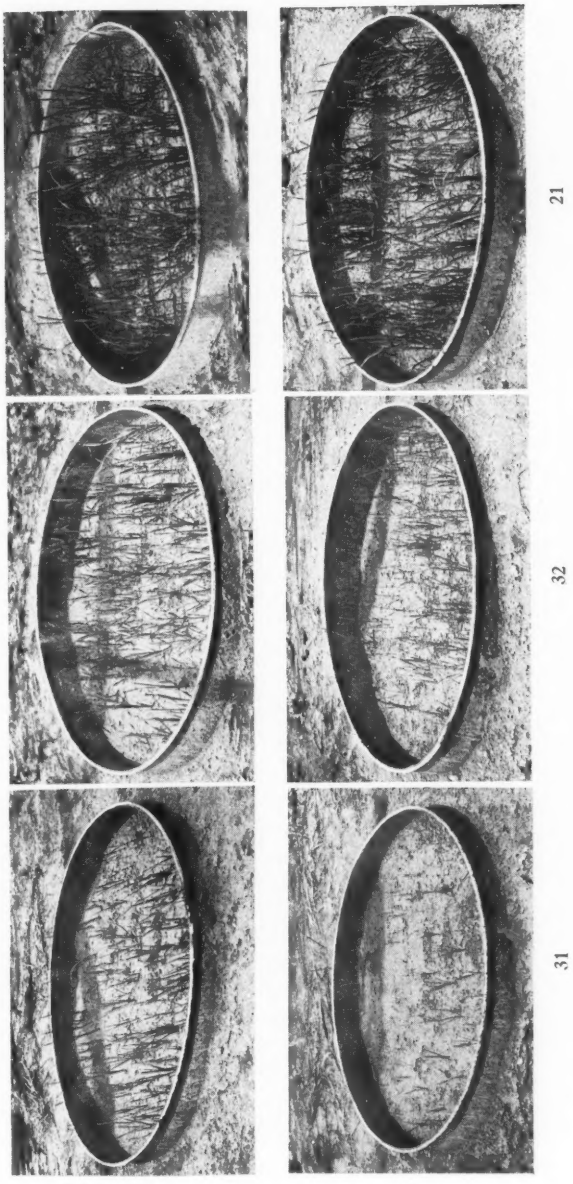


PLATE 2

COMPARISON BETWEEN EFFECT OF RESIDUAL PRECIPITATED CALCIUM CARBONATE AND THE NON-CARBONATE RESIDUALS FROM MAGNESIUM CARBONATE UPON TALL OAT GRASS DURING PERIOD OF FERTILITY FOLLOWING INITIAL PERIOD OF MAGNESIUM-INDUCED TOXICITY

Cylinders seeded April 7, 1914. Growth on cylinders 31 and 32 from fourth seeding, Oct. 26, 1914. Photo June 5, 1917.

Treatments per 2,000,000 lbs. soil. Series N

30 No treatment	18 30 tons stable manure	27 28,180 lbs. $CaCO_3$
31 $MgCO_3$ equivalent to 28,180 lbs. $CaCO_3$	32 $MgCO_3$ equivalent to 28,180 lbs. $CaCO_3$ and 30 tons stable manure	21 28,180 lbs. $CaCO_3$ and 43 tons stable manure.

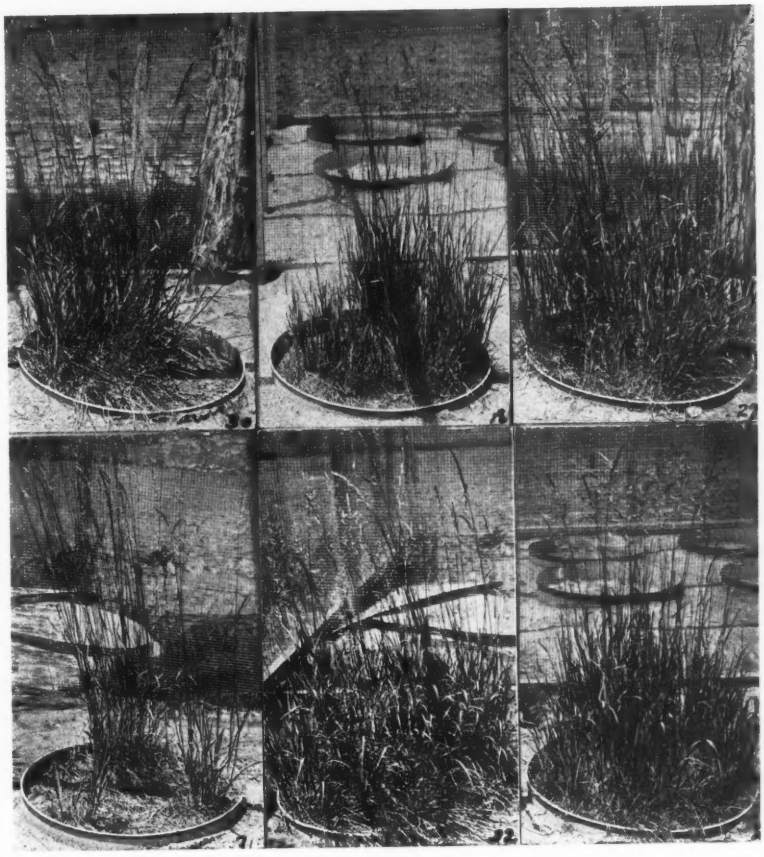


PLATE 3

SHOWING ABSENCE OF INITIAL MAGNESIUM-INDUCED TOXICITY, EARLY STAGE OF 1917 CROP

Treatments June 11, 1913, seeding April 7, 1914. Fourth seeding, responsible for growth in cylinders 31 and 32, made Oct. 26, 1914. Photo made March 19, 1917.

Treatments per 2,000,000 lbs. soil. Series N

30	18
No treatment	30 tons stable manure
31	32
$MgCO_3$ equivalent to 28,180 lbs. $CaCO_3$	$MgCO_3$ equivalent to 28,180 lbs. $CaCO_3$ and 30 tons stable manure

PLATE 3

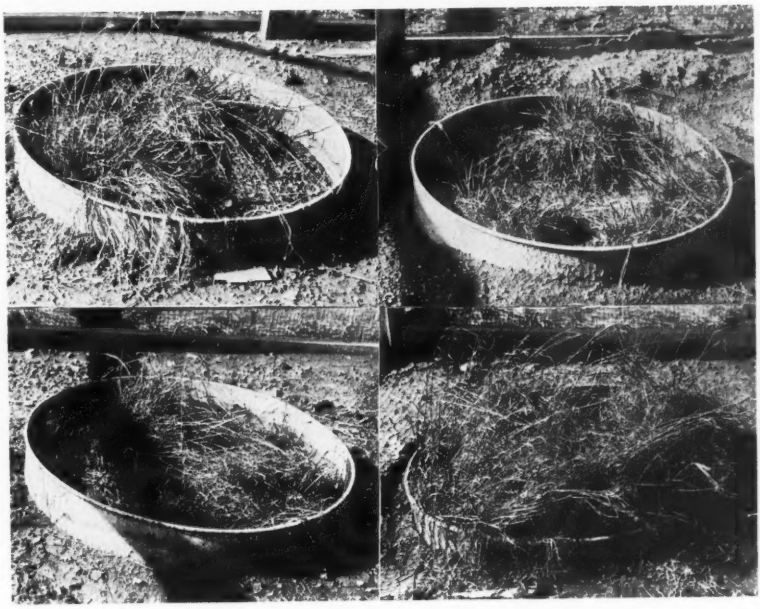


PLATE 4

SHOWING ABSENCE OF MAGNESIUM-INDUCED TOXICITY UPON COWPEA CROPS 1917

Seeded June 16. Harvested September 4, 1917.

Treatments per 2,000,000 pounds of soil. Series K.

30 No treatment	18 30 tons stable manure
31 $MgCO_3$ equivalent to 28,180 pounds $CaCO_3$	32 $MgCO_3$ equivalent to 28,180 pounds $CaCO_3$ and 30 tons stable manure

PLATE 4

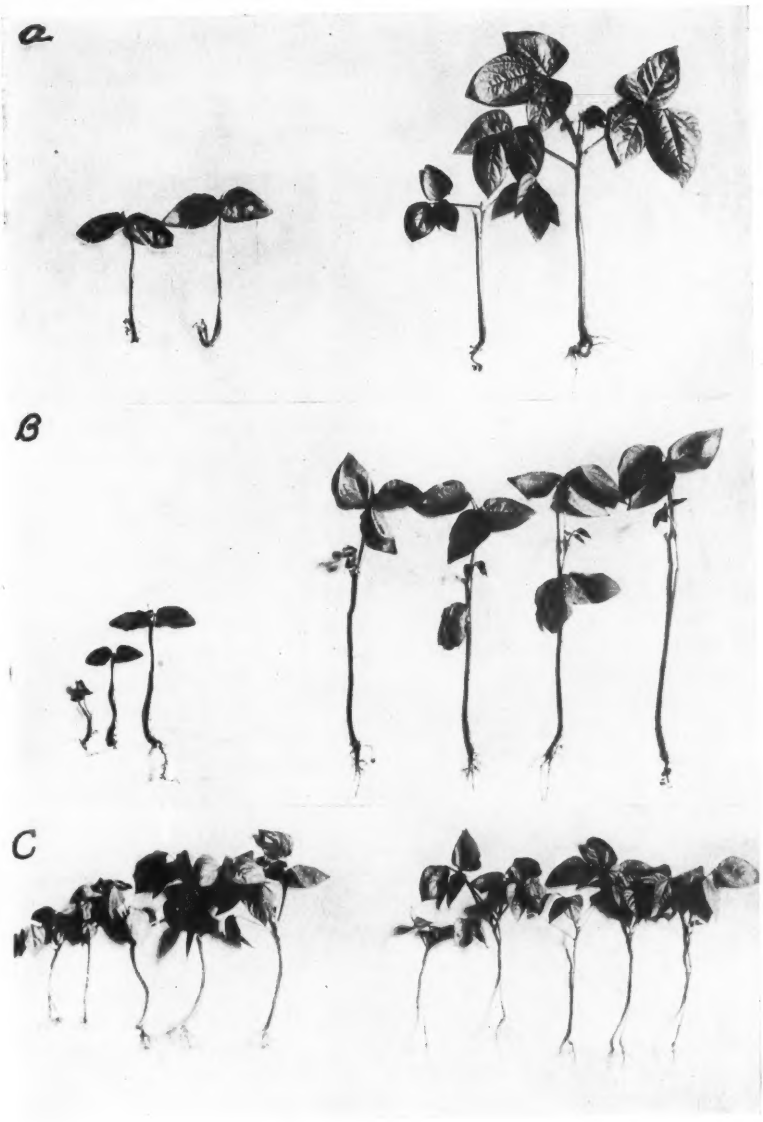
PLATE 5

SHOWING INITIAL TOXICITY INDUCED BY TREATMENTS OF $MgCO_3$ EQUIVALENT TO 28180 POUNDS $CaCO_3$ PER 2,000,000 POUNDS OF SOIL IN WAGNER POTS, AND ITS DISAPPEARANCE AFTER ONE YEAR

A. Treatment and seeding Aug. 3, 1918. Photo made in chlorotic stage Sept. 6, 1918.

B. Treatment Aug. 3, 1918, seeding Sept. 24, 1918. Photo made in chlorotic stage Oct. 23, 1918.

C. Treatment Aug. 3, 1918, seeding Sept. 3, 1919. Photo made in chlorotic stage, Oct. 2, 1919.


a
B
C

A NOTE ON SOIL REACTION STUDIES[1]

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Kentucky Agricultural Experiment Station

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For certain lines of investigational work of the Experiment Station, samples of soil have been collected from different plots of a number of the soil fertility experiment fields in the state. The lime requirement of these samples was determined shortly after collection by the Veitch and Hopkins methods. Lately the hydrogen-ion concentration has been determined by both the electrometric and colorimetric methods.

In the summer of 1916 Professor George Roberts made a field study of the growth of sweet clover in a section of the state where this is an important crop. The soils in this section are largely residual from predominatingly limestone formations. Up to the present but little attention has been paid to the reaction of the soils and the use of lime where necessary to insure successful growth of the crop. There have been wide variations in the success of the crop depending on the extent to which the original basic materials have been removed from the surface soil layers and their resulting reaction. In connection with this field study, samples of soil were taken from areas representing varying degrees of success of growth of the crop for further studies in the laboratory. Lime requirement determinations by the Veitch and Hopkins methods, and litmus paper and HCl reaction tests were made shortly after collection. Hydrogen-ion concentrations have also been determined lately by the colorimetric method.

The results from these reaction determinations, heretofore unpublished, are presented in this paper in connection with the yields of red clover in the field in the case of the experiment field plots and with the nature of the sweet clover growth indicated in the case of the sweet clover study samples.

For the experiment field soils only the pH values obtained by the electrometric method are given. The readings were made with a Leeds and Northrup student potentiometer. The hydrogen electrode vessels used were made in the laboratory. They were 3.4 cm. in diameter and 3.5 cm. in height, inside measurements. Clark hydrogen electrodes were used. The stopper carried the usual entrance and exit tubes for hydrogen, and connecting tube for calomel electrode. The hydrogen electrodes were cleaned and coated with platinum black, generally after every third determination. The calomel electrode was also made in the laboratory. Hydrogen from zinc was used for the most

[1] Contribution from the Department of Agronomy, Kentucky Agricultural Experiment Station. Published with permission of the Director.

TABLE 1

Reaction and clover yields on experiment field soils

PLOT NUMBER	TREATMENT*	REACTION (ELECTROMETRIC)	$CaCO_3$ REQUIREMENT PER ACRE		YIELD OF CLOVER HAY PER ACRE†
			Veitch method	Hopkins method	
		pH	*lbs.*	*lbs.*	*lbs.*
		Lexington Field			
403	R*	5.69	2800	0	4582
408	RL	6.23	1800	0	4293
401	RAP	5.78	3400	0	4478
406	RLAP	6.47	600	0	4268
		Russellville Field			
201		5.12	2340	240	1399
213	M	4.95	2480	360	1578
202	ML	6.92	0	0	2686
207	MLAP	6.80	0	0	3530
		Greenville Field			
201		5.07	1600	24	135
203	M	5.54	1000	76	843
202	ML	6.92	0	0	1555
207	MLAP	6.48	0	0	2831
		Mayfield Field			
201		4.68	3200	920	763
203	M	4.84	2600	820	978
202	ML	6.96	0	0	2848
204	MAP	4.59	3200	1120	1052
206	MLAP	6.75	0	0	3858

* R = residues
M = manure
L = limestone
AP = acid phosphate

The rotation used on these fields has been corn (with cover crop) soybeans, wheat, and clover. There are four series of plots to make possible the growth of all the crops each year. The applications of limestone and acid phosphate have been at the rate of 2 tons and 800 pounds respectively, per acre per rotation applied before the corn crop. On the fields where the manure treatments are used, the corn and wheat grain, the corn stalks, wheat straw, soybean and clover hay are removed. Beginning with 1916 manure has been applied once per rotation before the corn crops at the rate of 6 tons per acre, with the exception of the Berea field where the first application was in 1917. At the time of sampling, the plots had each had one application of manure. On the fields where the residue treatments are used, the corn, wheat and soybean grain and the clover hay are removed. The corn stalks and wheat and soybean straw are returned to the land. Fuller information as to the plan of conducting and results from these fields is given in Kentucky Experiment Station Bulletin 228 and Circular 123 (1, 2).

† Yields are averages of the four series usually from the beginning of the experiments through the 1922 yields, a period of 5–7 years on the various fields.

TABLE 1—*Continued*

PLOT NUMBER	TREATMENT	REACTION (ELECTROMETRIC)	$CaCO_3$ REQUIREMENT PER ACRE		YIELD OF CLOVER HAY PER ACRE†
			Veitch method	Hopkins method	
		pH	*lbs.*	*lbs.*	*lbs.*
		Berea Field			
101		4.91	1200	780	68
102	ML	6.40	0	0	660
104	MAP	4.77	2400	1220	706
106	MLAP	6.82	0	0	2058
		Fariston Field			
302		4.52	1400	1280	19
303	RL	6.29	0	0	323
308	RAP	4.62	1400	1220	842
311	RLAP	5.78	200	0	1599

TABLE 2

Reaction and clover yields on four experiment field soils

TREATMENT*	PLOT NUMBER	REACTION (ELECTRO-METRIC)	YIELD OF CLOVER HAY PER ACRE†	PLOT NUMBER	REACTION (ELECTRO-METRIC)	YIELD OF CLOVER HAY PER ACRE†
		pH	*lbs.*		*pH*	*lbs.*
	Russellville Field			*Mayfield Field*		
M	304	4.93	1578	307	4.95	978
ML	302	6.86	2686	302	7.35	2848
MAP	303	4.97	2299	304	4.80	1052
MRP	306	4.93	2791	305	5.13	1818
MLAP	307	6.94	3530	306	6.98	3858
MLRP	308	7.05	3433	308	7.19	3604
	Berea Field			*Fariston Field*‡		
M	203	4.50	374	207	4.45	113
ML	202	6.41	660	203	5.82	323
MAP	204	4.35	706	208	4.63	842
MRP	205	4.57	717	205	4.56	794
MLAP	206	6.20	2058	211	5.85	1599
MLRP	207	5.99	1849	214	5.70	811

*M = manure
L = limestone
AP = acid phosphate
RP = rock phosphate

Limestone and acid phosphate applications same as indicated in table 1. Rock phosphate applied at rate of 1600 pounds per acre per rotation before the corn crop.

† The clover yields are the same as those in table 1 except for the addition of the yields on the RP and LRP plots.

‡ Crop residues instead of manure have been used on the Fariston field.

part. Certain readings were repeated with tank hydrogen from the Will Corporation prepared for hydrogen-ion work. The readings were in good agreement.

Ten grams of soil and 15 cc. of water were used per reading. The vessels were hand shaken in so far as additional agitation was necessary over that given by the bubbling of hydrogen. The readings on most of these soils were made by Wm. M. Phipps, a student doing some special work in soils, as well as by the writer. Differences in readings due to the personal equation were not important. A difference of pH .05 was the maximum variation in duplicate readings. The duplicate readings were made at the same time using two hydrogen electrode vessels. No difficulty was experienced in getting this agreement when the outfit was in satisfactory working condition. Fifteen to twenty minutes was the time usually required for constant potential to be reached. Corrections were made for variations in temperature.

With the exception of the Lexington samples, the samples reported in table 1 were taken in October, 1920. The Lexington samples were taken in December, 1920. At the time of sampling, the plots were in clover seeded in the preceding spring, in wheat. The Hopkins and Veitch determinations were made at various times from December, 1920, to March, 1921. The hydrogen-ion concentration readings were made for the most part in August, 1922.

Soil samples were also taken from the Mayfield and Russellville fields in July, 1921, and from the Berea and Fariston fields in December, 1921. At this time the Russellville, Mayfield, and Berea fields were in clover, seeded in small grain in the preceding spring. The Fariston field had been in corn in 1921 and was in a cover crop of rye at the time of sampling. The hydrogen-ion concentration of these samples was determined electrometrically by J. F. Freeman, graduate student, in September, 1922, using the same apparatus and procedure as in the preceding determinations. The results are given in table 2. For the most part differences in time of sampling and in series sampled have had but little effect on the pH values as shown in tables 1 and 2. These values were determined after the samples had been in the air-dry condition in the laboratory for some time. It is possible that readings on the fresh samples might have been somewhat different.

Limestone is the important fertilizer treatment in affecting hydrogen-ion concentration and resulted in pH values of 6.5–7.0, except on the Lexington and Fariston fields. It is noteworthy that the Lexington field where the unlimed soil gives the highest pH value is the one where, on the whole, limestone has been of least effect in raising pH value. This may be accounted for by assuming it to be a more highly buffered soil because of its larger organic matter content. Where applied without limestone, rock phosphate has given considerably larger yields of clover as well as of other crops than has acid phosphate, but the effect of the two materials on hydrogen-ion concentration has been very slight if any.

TABLE 3

Sweet clover study soils arranged in order of hydrogen-ion concentration of surface soils

SAMPLE NUMBER*	REACTION (COLORIMETRIC METHOD)	$CaCO_3$ REQUIREMENT		REACTION FROM LITMUS PAPER TEST§	EFFERVESCENCE ON ADDING HCl	NATURE OF SWEET CLOVER GROWTH
		Veitch method	Hopkins method			
	pH	*lbs.*	*lbs.*			
2	7.63	*alk.*	100	neut. to alk.	strong	good
2a†	7.63	*alk.*	160	neut. to alk.	strong	
1	7.00	*alk.*	*alk.*	neut.	strong	good
1a	5.65	....	60	sl.§ acid	doubtful	
15	7.00	*alk.*	10	neut.	medium	good
15a	5.15	1365	440	acid	none	
18	6.35	*alk.*	70	neut.	medium	good
18a	7.40	*alk.*	40	neut.	medium	
9	6.40	*alk.*	40	.....	.....	good
21	6.00	....	40	v. sl. acid	medium	good
21a	....	....	80	sl. acid	strong	
22	5.70	....	40	v. sl. acid	medium	good
22a	7.63	....	50	neut.	strong	
4	5.60	*alk.*	90	neut.	none	good
4a	4.90	1911	680	neut.	none	
7	....	*alk.*	120	neut.	none	good
7a	5.30	1465	....	v. sl. acid	none	
10	5.40	1638	40	neut.	medium	good
10a	5.20	4641	1580	acid	medium	
6	5.45	637	70	neut. to alk.	none	poor
6a	4.90	5369	4580	str. acid	slight	
11	5.05	910	140	sl. acid	none	almost none
11a	5.45	2366	1080	acid	none	
12	4.95	1911	500	acid	none	none
12a	5.15	....	1360	str. acid	none	
8‡	....	2912	1300	acid	none	poor
8a	4.70	5005	4600	acid	none	
8b	4.90	7553	7000	acid	none	
8c	7.80	*alk.*	40	neut. to alk.	medium	
16	4.83	1911	480	acid	none	poor
16a	4.95	4823	3980	str. acid	none	
19	4.83	2366	1180	acid	none	none
19a	4.83	6370	6460	acid	none	
20	4.83	....	920	sl. acid	none	none
20a	4.83	....	4760	acid	none	
23	4.83	....	720	acid	none	none
23a	4.80	....	3960	acid	none	
3	4.45	5915	4240	sl. acid	none	none
3a	4.60	9646	11040	med. acid	none	
17	4.28	4550	2000	acid	none	none
17a	4.75	6734	8080	acid	none	

* Samples no. 1–19 are from Pendleton County and no. 20–23 from Harrison County. Sweet clover is a very important crop in these counties. It is grown for pasturage, hay and for seed production. The soil, largely of limestone origin, is a brownish yellow to yellow heavy silt loam to clay loam surface, and a heavy yellow clay loam under soil. The limestone present in the soils is for the most part fragments from the original rock formation and does not represent material added in liming.

† The letter "a" indicates corresponding subsurface sample, 6–18 inches.

‡ Surface, 0–6 inches; a, 6–12 inches; b, 12–18 inches; c, 18–24 inches.

§ Sl. = slightly, v. = very, str. = strongly.

The hydrogen-ion concentration of the sweet clover study samples were determined in January, 1923. The determinations were entirely by the colorimetric method. Clark and Lubs methyl red, brom-cresol purple, brom-thymol blue, and phenol red were the indicators used. The Clark and Lubs standard buffer solutions were also used with the exception of those in the lower range where the acid potassium phthalate was replaced by Sorensen's HCl-citrate solutions. The standards as made up covered the range in steps of about pH 0.3. It was necessary to adjust the reaction of the indicator solutions with small amounts of weak acid or alkali to make the colorimetric readings correspond with the electrometric as tested on the experiment field soils. The colorimetric extracts were made by shaking in a test tube 10 gm. of soil and 15 cc. of water at intervals for 30 minutes, then throwing on filter and pouring back till clear. The reported readings are averages of duplicate readings agreeing within pH 0.10. The pH values as well as the results of the other work are reported in table 3.

These results have not been presented as a basis for any conclusions. The close correlation between limestone treatment or presence of limestone material in soil and hydrogen-ion concentration, however, is to be noted; the reaction of soils treated with limestone or containing natural limestone fragments usually ranged from pH 6.5 to pH 7.5, the untreated acid soils ranged from pH 4 to pH 5.

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THE DESTRUCTION OF PENTOSANS BY MOLDS AND OTHER MICROÖRGANISMS[1]

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The pentoses are five-carbon-atom sugars found widely distributed in nature, chiefly as polysaccharides of high molecular weight which are known as pentosans. Next to cellulose they are the most widely distributed constituent of plant life comprising in some cases as much as 25 per cent of the weight of the plant. A study of the decomposition of these substances in nature by Fred, *et al* (3, 4) indicates that there are presentevery where microörganisms which are able to break down the pentoses and related compounds.

Among the numerous microörganisms involved in the decay of organic matter, none possess a wider range of fermentation power than the molds. They ferment carbohydrates, proteins, fats, alcohols, organic acids, etc., but the destruction of the pentoses and pentose-yielding substances by these forms of life has been investigated to only a limited extent. The rapidity with which pentosan-containing materials are broken down in nature suggests the possibility that fungi play an important rôle in their destruction.

The value of pentose sugars as sources of carbon for fungi has been presented in a recent publication by the authors (8). Among the twenty-five species of molds studied, sixteen were found to ferment the pentoses with rapidity. Most of the remaining nine cultures grew slowly and three or four produced only a few mycelian threads. The best fermenters were found among the *aspergilli* and *penicillia* species although a number of molds of these types attacked the pentoses but slowly. The *Mucors*, *Rhyzopus nigricans*, and *Cunninghamella* were also found to be slow fermenters. Four or five days sufficed for the complete destruction of 4 per cent solutions of xylose and arabinose with the most active forms. Compared with glucose, the two pentoses were fermented somewhat less rapidly but the difference was slight. Carbon dioxide and mycelium represented the major portion of the sugars consumed. In 10 per cent concentrations of xylose a considerable quantity of acid was developed and good qualitative tests for citric acid were obtained.

In 1915 Hawkins (5) found that the percentage of furfural-yielding material in the decayed part of an apple inoculated with *Glomerella cingulata* was considerably less than in the sound portion. He also found that xylan and araban are not as readily available for the fungus as the pentose sugars themselves. Tochinai (9) found that *Fusarium lini* can utilize araban. In view of the fact that so little is known concerning the destruction of pentosans by the more common molds, an investigation was undertaken along this line.

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METHODS

The molds and nutrients used in this work were the same as those reported in a previous publication (8). From the twenty-five species there studied eight of the most vigorous pentose fermenters were selected for this investigation. The nutrient solution had the following composition:

	cc.
M ammonium nitrate	250
M monobasic potassium phosphate	100
M magnesium sulfate	40
M ferric chloride	2
Distilled water	608

The individual salts were made up in quantities sufficient to cover a series of experiments. Each stock solution was kept in a separate flask and was combined with the others as needed.

TABLE 1

The effect of salts on the determination of pentosans in corn stover

TREATMENT OF STOVER	PENTOSAN CONTENT
	per cent
Water	19.9
25 cc. of salt solution	14.6
25 cc. of salt solution minus NH_4NO_3	20.4
25 cc. of salt solution minus $MgSO_4$	13.7
25 cc. of salt solution previously distilled with MgO to drive over the NH_3	12.8
25 cc. of salt solution with $NaNO_3$ replacing NH_4NO_3	13.6
25 cc. of salt solution with NH_4Cl replacing NH_4NO_3	20.6
25 cc. of salt solution washed with water on asbestos	19.5
25 cc. of salt solution washed with water on asbestos	20.3

DESTRUCTION OF PENTOSANS OF CORN FORAGE

Field corn at the silage stage of maturity was dried and ground to a fine powder which would pass through a 100-mesh sieve. This material was further dried at 105°C. and accurately weighed amounts, in the neighborhood of one gram, were placed in 150-cc. Pyrex Erlenmeyer flasks containing 25 cc. of the salt solution, and were sterilized for about an hour at 20 pounds of steam pressure. They were then inoculated with the spores of pure cultures of molds and incubated at 28°C. The fungi grew well on this medium and in a few days the surface of the culture solutions was covered with well developed mycelia. At various intervals the entire contents of some of the flasks were removed and analyzed for pentosans by the method described by Kröber (7).

It was immediately discovered that the nitrates remaining in the culture solution interfered in the pentosan determination by destroying a large part of the furfural. The results in table 1 show the effect of various salts upon the determination of pentosans. The oxidizing effect of the small amount of HNO_3

secured from the reaction of the HCl upon the NH_4NO_3 is clearly shown by a decrease of 30 per cent in the furfural obtained from a distillation performed under such conditions as compared with a normal acid distillation. $NaNO_3$ when replacing NH_4NO_3 acts in a similar manner, whereas NH_4Cl in the solution is without effect. In one case the ammonia was first removed by distillation with MgO but this procedure is not necessary because ammonia does not interfere in the reaction. However, it was found that the corn stover could be transferred to an asbestos filter and the salts removed by washing with distilled water. The residue and the asbestos could then be put in a distilling flask and the pentosans determined in the usual manner. Under these conditions there was practically no loss of furfural-yielding material.

Table 2 gives the percentage of pentosans remaining in the cultures at various intervals. The figures in the last column show that *Aspergillus fumigatus* destroys the pentosans in the corn forage to a greater extent than any of the other molds studied; over 50 per cent disappeared in 142 days. When corn forage was kept in a silo and molds did not develop it was found that about 25–30 per cent of the pentosans were destroyed. The molds were, therefore, about twice as active in the destruction of pentosans as the microörganisms contained

TABLE 2

Destruction of the pentosans of corn forage by molds

CULTURE NUMBER	MOLD	PENTOSANS REMAINING AFTER						AMOUNT DESTROYED
		0 days	15 days	39 days	46 days	63 days	142 days	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
15	*Aspergillus flavus*	20.0	17.1	14.4	....	13.4	11.8	40.1
16	*Aspergillus fumigatus*	20.0	16.2	15.3	....	10.6	9.4	53.0
5	*Aspergillus niger*	20.0	15.1	15.4	14.2	....	11.1	44.5
24	*Aspergillus oryzae*	20.0	....	....	....	....	12.4	38.0
12	*Aspergillus repens*	20.0	....	....	....	....	13.8	31.0
6	*Penicillium glaucum*	20.0	17.8	15.8	....	15.0	13.4	33.0
9	*Cunningham. sp.*	20.0	....	....	....	....	11.5	42.5
8	*Rhizopus nigricans*	20.0	....	....	....	....	14.1	29.5

in silage. The cultures grew well at first because a large amount of readily available carbohydrate other than pentosans and cellulose is found in corn forage. A larger amount of the pentosans are destroyed during the first few weeks than during the latter part of the fermentation period.

DESTRUCTION OF THE PENTOSANS OF RYE STRAW

The rye straw cultures were prepared in the same manner as those grown on corn forage and inoculated with the same molds. Similar cultures were also set up and inoculated with 1 cc. of a soil suspension, and others with 1 cc. of a suspension of cow feces. The organisms did not grow as well as on corn stover, due probably to the lack of readily available carbohydrate such as sugars or

starch. After a month's incubation a light mycelial growth had developed upon the surface of the mold cultures, while the surface of the cultures inoculated with soil suspension and cow feces became covered with a green mold growth. At various intervals the entire contents of some of the flasks were transferred to an asbestos filter, washed with distilled water to remove nitrates, and the pentosans determined in the usual manner.

The last column in table 3 indicates that 33–38 per cent of the total pentosans of the rye straw has been utilized by the fungi in 300 days. It is of interest to note that the pentosans in corn forage are destroyed much more rapidly than the pentosans of the rye straw. A comparison of the soil and cow feces sus-

TABLE 3

Destruction of pentosans of rye straw by molds

CULTURE NUMBER	MOLD	PENTOSANS REMAINING AFTER					AMOUNT DESTROYED
		0 days	29 days	103 days	175 days	300 days	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
15	*Aspergillus flavus*	26.2	24.3	22.5	21.9	16.2	38.1
16	*Aspergillus fumigatus*	26.2	21.3	19.3	17.4	17.0	35.1
5	*Aspergillus niger*	26.2	22.1	20.5	18.4	17.3	33.9
6	*Pencillium glaucum*	26.2	22.3	20.1	19.9	16.5	36.6
	Soil suspension	26.2	26.4	19.4	18.4	17.0	35.1
	Cow feces suspension	26.2	26.0	20.2	19.6	17.5	33.2

pensions shows that the pure mold cultures can destroy the pentosans as rapidly as can a mixed culture of molds and bacteria. These results prove that the fungi can utilize the pentosans of corn forage and rye straw but not nearly so well as the free pentoses dicussed in a previous paper (8) where a complete destruction was secured within a week.

DESTRUCTION OF THE PENTOSANS OF WOOD BY MOLDS

In these experiments 1 gram of the finely ground alder, poplar, or birch wood was introduced into 150-cc. Erlenmeyer flasks with 25 cc. of the nutrient salt solution. The ammonium nitrate was replaced by an equivalent amount of ammonium acetate. The flasks were sterilized and inoculated as usual and incubated at 28°C. The cultures would not grow so 1 cc. of a 10 per cent sucrose solution was added to each flask but the molds would not grow even under these conditions. A few of the cultures were analyzed afterwards but the total pentosans had not decreased to any appreciable extent. Evidently some substance was extracted from the woods which inhibited the growth of the molds.

DESTRUCTION OF PENTOSANS IN SOIL

In studying the activity of molds in the destruction of the pentosans of various woods it was found that the molds would not grow on a liquid synthetic

medium containing these woods. It was therefore deemed advisable to repeat the experiment duplicating as closely as possible conditions existing in nature.

About a ton of virgin silt loam soil was obtained from Rusk County, Wisconsin. Samples of the brush and young wood growth were taken from the sample place where this virgin soil was obtained. This brush consisted of both green and dry poplar, birch, and tag alder. The soil was sifted through a screen to remove all roots, moss, sticks, etc., and equal weights of soil were placed in 4-gallon jars. The moss was weighed and the weight of moss corresponding to the weight of soil contained in each jar was computed. This gave 300 gms. of moss to be added to each jar to give the soil the same content of moss as would be found under field conditions.

The wood was cut up into pieces 3–6 inches long and $\frac{1}{8}$–2 inches in diameter. These pieces were divided into two groups according to thickness. Those under three-fourths of an inch in diameter were designated as "fine" wood, and those above this size were called "coarse" wood. Various weights of these woods were mixed with the different soils. The moisture content was brought up to 20 per cent and maintained at this percentage during the course of the experiment. All the jars were kept in the green house at approximately 30°C.

About two months after the wood had been placed in the soil, oats and red clover were planted in each jar, and two months after planting both top and root of the crops were removed from the jars. The soil was sifted and the residue moss and wood, after the removal of all adhering particles of soil, were dried and weighed. The moss and wood were ground separately to a fine powder and their pentosan content determined in the usual manner. This data is given in table 4.

Only a small portion of the moss could be recovered from the soil and in some cases it had disappeared entirely. It was either completely destroyed or disintegrated to such an extent that it passed through the sieve and so remained with the soil. This probably accounts to a large extent for the increase in pentosans in the soil at the end of the experiment.

Approximately one half of the wood added to the soil was destroyed. The greatest destruction took place in the case of poplar. As would be expected the loss was greater for the fine wood than for the coarse. More than half, 44–67 per cent, of the total pentosans contained in the wood and moss were destroyed. The average loss for the 8 jars was 58.1 per cent.

In table 5 is given a comparison between the destruction of wood and the destruction of one of the wood constituents, the pentosans. An average of over 61 per cent of the poplar wood was destroyed during the course of the experiment; over 50 per cent of the birch wood and about 35 per cent of the alder wood. The pentosans in the various woods were destroyed to the extent of 69 per cent for popular, 60 per cent for birch, and 47 per cent for alder. It is especially noticeable that in every case the pentosans have disappeared more rapidly than the cellulose, lignin, or other woody constituents, proving that the pentosan complex is more susceptible to the natural processes of decay than the other constituents of the wood.

TABLE 4

The destruction of pentosans by the microörganisms of the soil

MATERIAL	BEFORE PLANTING			AFTER CROPPING			LOSS OF PENTOSANS	
	Weight	Pentosans		Weight	Pentosans			
	gm.	*per cent*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*per cent*
Jar 5:								
Soil	11,622	0.102	11.85	11,622	0.110	12.78		
Moss	300	9.20	27.60	44	6.31	2.78		
Coarse alder	225	22.34	50.27	124	18.07	21.41		
Total			88.72			36.97	52.75	58.8
Jar 6:								
Soil	11,622	0.102	11.85	11,622	0.109	12.67		
Moss	300	9.20	27.60	26	5.59	1.45		
Coarse alder	225	22.34	50.27	148	19.56	28.95		
Total			89.72			42.07	46.65	52.0
Jar 9:								
Soil	11,622	0.102	11.85	11,622	0.110	12.78		
Moss	300	9.20	27.60	None	.....	.....		
Fine alder	450	22.34	100.54	290	16.68	48.37		
Total			139.99			61.15	78.84	56.3
Jar 10:								
Soil	11,622	0.102	11.85	11,622	0.123	14.30		
Moss	300	9.20	27.60	23	2.52	0.58		
Fine alder	450	22.34	100.54	326	19.40	63.24		
Total			139.99			78.12	61.87	44.2
Jar 25:								
Soil	11,622	0.102	11.85	11,622	0.130	15.11		
Moss	300	9.20	27.60	28	5.68	1.59		
Fine poplar	450	21.80	98.10	160	17.28	27.65		
Total			137.55			44.35	93.20	67.8
Jar 26:								
Soil	11,622	0.102	11.85	11,622	0.119	13.83		
Moss	300	9.20	27.60	19	5.18	0.98		
Fine poplar	450	21.80	98.10	190	16.70	32.73		
Total			137.55			47.54	90.01	65.4

TABLE 4—*Continued*

MATERIAL	BEFORE PLANTING			AFTER CROPPING			LOSS OF PENTOSANS	
	Weight	Pentosans		Weight	Pentosans			
	gm.	*per cent*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*per cent*
Jar 17:								
Soil	11,622	0.102	11.85	11,622	0.138	16.04		
Moss	300	9.20	27.60	.....	.....	.....		
Fine birch	450	24.60	110.70	218	23.34	50.88		
Total			150.15			66.92	83.23	55.4
Jar 23:								
Soil	11,622	0.102	11.85	11,622	0.139	16.15		
Moss	300	9.20	27.60	.....	.....	.....		
Fine birch	450	24.60	110.70	212	17.16	36.38		
Total			150.15			52.53	97.62	65.0

TABLE 5

Comparative destruction of wood and pentosans

WOOD	WOOD DESTROYED		PENTOSANS DESTROYED	
	gm.	*per cent*	*gm.*	*per cent*
Coarse alder	101	44.8	28.9	57.4
Coarse alder	77	34.2	21.3	42.4
Fine alder	160	35.6	52.2	51.9
Fine alder	124	27.6	37.3	37.1
Fine birch	232	51.5	59.8	54.0
Fine birch	238	52.9	74.3	67.1
Fine poplar	290	64.4	70.5	71.8
Fine poplar	260	57.8	65.4	66.6

PENTOSAN CONTENT OF MOLDS GROWN ON VARIOUS MEDIA

It has long been known that the mycelium of most molds yields furfural when distilled with hydrochloric acid showing the presence of a pentosan complex. Wichers and Tollens (10) in 1910 found that the mycelium of fifteen species of xylophagous wood-destroying fungi contained 2.5–6.7 per cent of pentosans. In 1911 Dox and Neidig (2) found 0.86–1.17 per cent pentosans in Penicillium glaucum and Aspergillus niger. They found no purins present. Nucleoproteins, therefore, could not be regarded as the source of the pentoses. They concluded that pentosans are normal constituents of the cell structure of lower fungi and are formed independent of the presence of pentose complexes in the culture media. Ishida and Tollens (6) in 1911 found 2–5 per cent of pentosans in the fungi and 1–2 per cent methyl pentoses. In 1901 Bendix obtained a pentose from tuberculosis bacilli by boiling the dried bacilli with 5 per

cent HCl. The pentose was found to be contained in the nucleo proteins of the bacilli. A mixture of fecal bacteria cultivated in urine and the bacilli of diphtheria both showed the pentose reactions.

Culture solutions were made containing 50 cc. of 4 per cent sucrose and 50 cc. of the nutrient salt solution. These were sterilized for an hour under 20 pounds of steam pressure and 10 of each inoculated with the spores of the various fungi. All of the cultures grew well and produced thick pads, and at the end of seven days a test showed that all the sugar had disappeared. One-half of the cultures were then removed, filtered, and the mold growth thoroughly washed with distilled water. The material was then dried, ground to a fine powder, dried

TABLE 6

Pentosan content of the mold growth

CULTURE NUMBER	MOLD	SUGAR	AGE	DRY WEIGHT AT 105°C.	PENTOSAN	
			days	*gm.*	*mgm.*	*per cent*
15	Aspergillus flavus	Sucrose	7	3.010	26.1	0.84
		Sucrose	30	1.784	15.7	0.85
		Xylose	10	2.741	36.3	1.32
16	Aspergillus fumigatus	Sucrose	7	3.105	27.5	0.88
		Sucrose	30	2.154	16.8	0.78
		Xylose	10	2.621	29.2	0.98
5	Aspergillus niger	Sucrose	7	2.855	21.6	0.76
		Sucrose	30	2.783	19.4	0.68
		Xylose	10	1.624	17.7	1.09
24	Aspergillus orzyae	Sucrose	7	2.872	23.8	0.83
		Sucrose	30	1.241	10.8	0.87
6	Penicillium glaucum	Sucrose	7	2.680	26.8	1.00
		Sucrose	7	2.678	30.8	1.15
		Sucrose	30	1.790	21.1	1.18
		Sucrose	30	2.000	26.0	1.30
		Xylose	10	2.556	36.6	1.43

to constant weight at 105°C. and analyzed for pentosans. When the remaining half of the sucrose cultures were a month old they were treated in a similar manner. These cultures appeared dead from the lack of carbohydrate and had apparently decreased in weight.

At the same time an experiment was undertaken to determine whether the organism could deposit more pentosan within its mycelium if grown on a nutrient medium containing xylose as the only source of carbon. Therefore five flasks containing 100 cc. of about 2 per cent crude xylose for each mold was set up in the same manner as was done with the sucrose cultures. After 10 days of incubation a sugar test showed that all the xylose had been assimilated

and the cultures were then filtered and thoroughly washed with distilled water. The combined filtrate did not give any test for reducing sugars. The mold growths were treated as described above. The data are given in table 6.

Apparently pentosans are deposited within the mycelium of the fungi independent of any pentose complex in the culture media. If the fungi are grown on a pentose as a source of carbon a larger amount of furfural-yielding material is deposited within the cells of the mycelium. After all the carbohydrate in the medium is assimilated, the fungus apparently metabolizes some of the pentosan complex which is found in its mycelium as there is a distinct decrease in the amount of pentosans in the mold after it has existed for several weeks on a carbohydrate free medium.

SUMMARY

In studying the activities of molds and other microörganisms in the destruction of pentosans it was found that the molds could destroy about 50 per cent of the pentosans of corn forage within 100 days; about 35 per cent of the pentosans of rye straw in 300 days. These organisms would not grow on a synthetic liquid medium with wood as a source of carbon nor did the addition of sucrose result in any growth.

When placed in soil on which a crop was grown, the pentosans of wood were rapidly destroyed. In six months about 60 per cent of the total pentosan content of the wood was destroyed. A larger percentage of pentosans was destroyed than of the wood itself, showing that pentosans are more readily attacked than the cellulose, lignin and other constituents of the wood.

It was found that pentosans are present as a natural cell constituent of the common fungi and are deposited in the mycelial growth to an extent of about 1 per cent, even in the absence of any pentose material in the culture medium. This mycelial pentosan complex can later serve as a source of carbohydrate for the organism. When grown on a medium containing xylose the fungi deposit more pentosans in their cells than when grown on sucrose.

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MICRO-SAMPLING FOR THE DETERMINATION OF DISSOLVED OXYGEN[1]

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New Jersey Agricultural Experiment Stations

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The problem of aeration of the substratum as affecting the development of plants in artificial cultures has been the subject of investigation in this laboratory during the past two years (1, 2, 4). Since it has been found desirable to study the fluctuation of dissolved oxygen in the substratum under the various conditions of these studies, the development of a method of sampling the comparatively small quantities of liquids available has been the subject of special study.

The Mikro-Winkler method suggested by Lund (3) was used in this work. It should be noted that the usual recommendations for sampling emphasize the necessity of protecting the sample to be tested from contact with the atmosphere or from the entrainment of bubbles of air within the sampling device. For the purpose to which it is commonly put, however, as in the case of studying the content of oxygen in large bodies of water, rivers, etc., the size of the sample taken is greatly in excess of that possible under the present conditions. For this reason the following procedure has been developed for use in sampling from such small sources as from one- or two-liter culture vessels with minimum exposure of the sample to the air while sampling and during its subsequent treatment.

In figure 1, the apparatus is shown at *A* ready to draw the sample. The bottle may hold any amount desired, usually about 25–30 cc. The inlet tube *a* is dipped into the solution and suction is applied at the tube *d* to which, for convenience, a piece of rubber tubing may be attached. After filling the bottle, care should be taken to draw out any air bubbles as quickly as possible. The flow is then allowed to continue through the system, the rubber tube acting as the long arm of a siphon, until the volume of the bottle has passed through or until the solution which first filled the bottle is entirely replaced with fresh solution that has not been exposed to the air. After this the pinch cock *C* is closed with the system completely filled. The sample is then ready to be treated with the reagents.

The manner in which this is done without exposing the sample to the air is shown in figure 1 at *B*. The part *a* of the inlet tube is removed and the

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portion *b* remains in the bottle, being permanently fixed in the stopper. The outlet tube *d* is also removed at the same time, care being taken to keep the bottle in an upright position during the process so that no air may enter through the opening in the stopper. Then by inserting into the opening of the stopper at *r* a 1-cc. pipette with graduation intervals of 0.01 cc. the necessary reagents may be readily added, the flow from the pipette being controlled by the pinch cock at *C*.

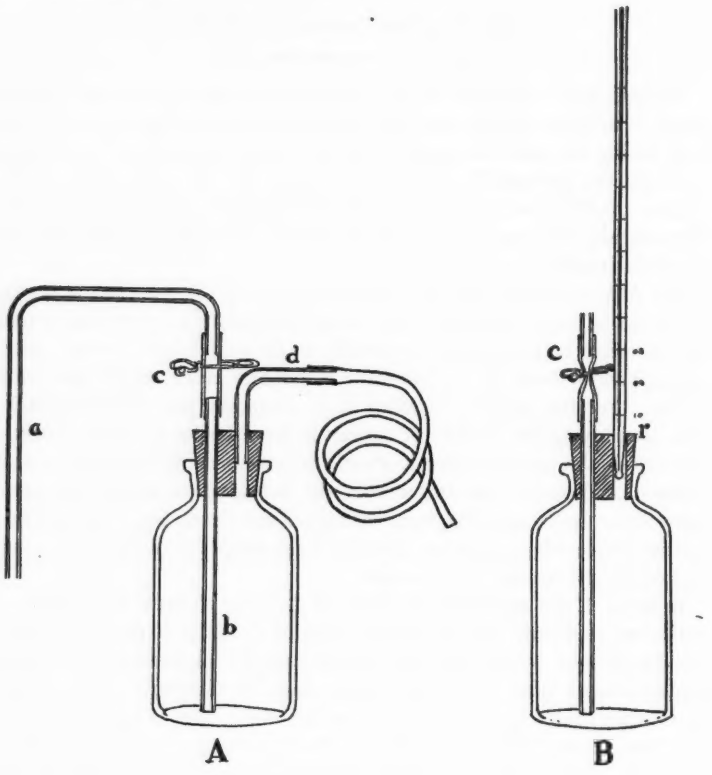

FIG. 1. DIAGRAM OF SAMPLING APPARATUS FOR OXYGEN DETERMINATIONS

After the addition of the reagents and their proper mixing with the sample by rotating the bottle the stopper may be removed. It is important to keep the pinch cock *C* closed while removing the stopper so that the solution displaced by the addition of the reagents and still remaining in the tube may not drop back into the treated sample. After the removal of the stopper from the bottle the titration may be carried out in the usual manner.

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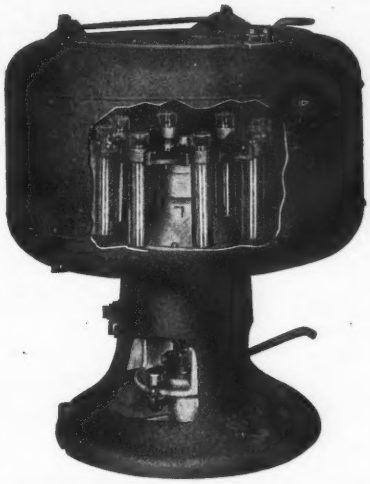

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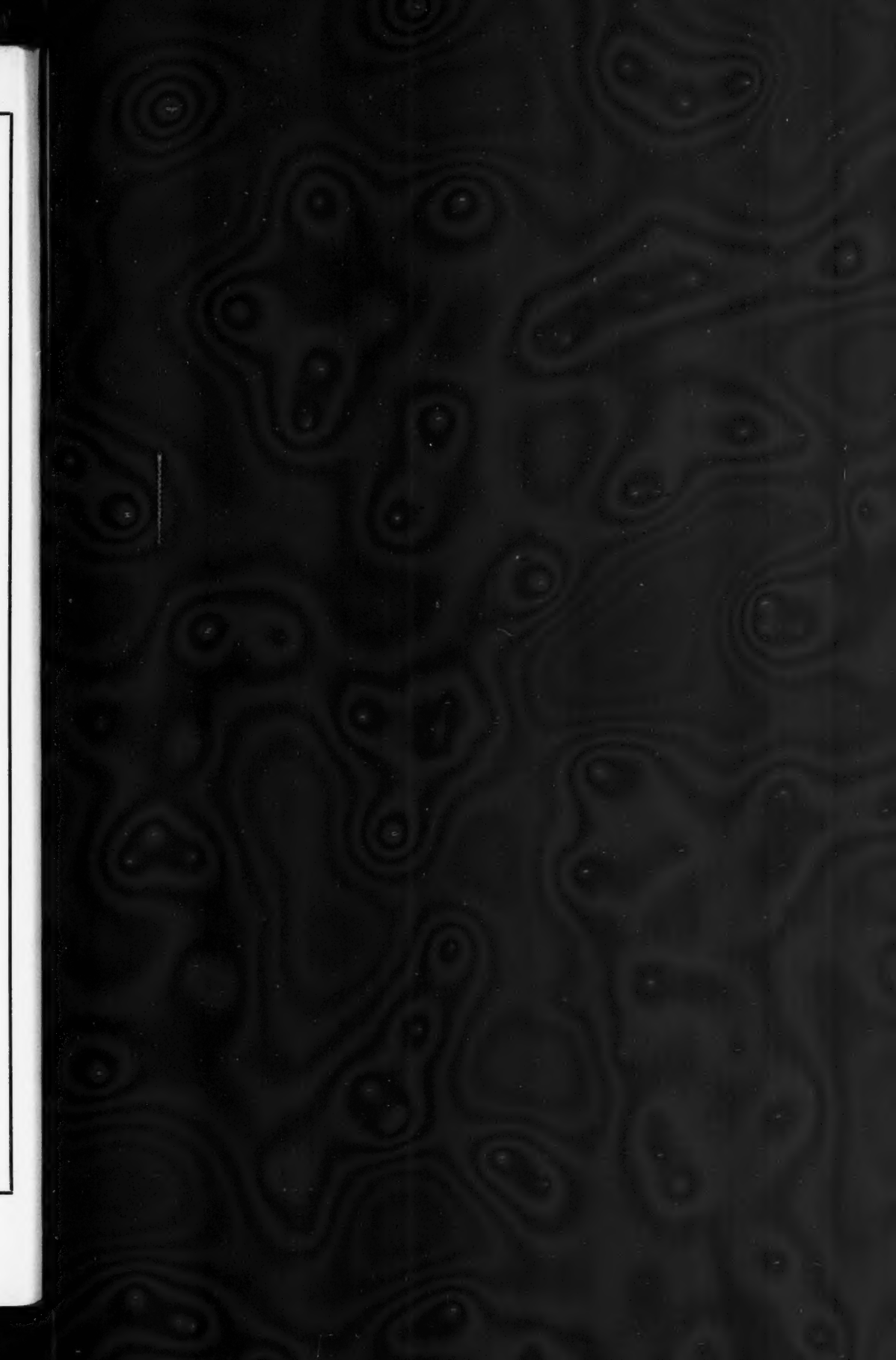

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